MACROINVERTEBRATE COMMUNITY ASSESSMENT OF THE MID-REACHES OF THE BUFFALO NATIONAL RIVER

By

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ABSTRACT

Staff associated with the Buffalo National River have conducted water-quality monitoring on the river and its tributaries since 1985, and analysis indicates that elevated nitrogen concentrations occur within the mid-reaches. A comparative study of the river continuum concept (RCC) model and the Buffalo River found that macroinvertebrate community indices for diversity and richness for the mid-reaches were much less than the upper and lower portions of the river. Declines in community diversity and richness correlated strongly with increased nitrate concentrations during the spring. The goal for this investigation was to examine relationships among water-quality, physical habitat, and macroinvertebrate communities within the mid-reaches of the Buffalo River to better understand the interactions that may explain losses in community richness and diversity. A total of 59,305 organisms representing 5 phyla, 8 classes, 14 orders, and 48 families were collected and identified. Correlative evidence generated during this investigation corroborates the results found by Bryant (1997), and suggests that declining water-quality and increasing densities of Corbicula are the two disturbances that are most likely to be responsible for shifts in the macroinvertebrate community's species composition. Long-term biomonitoring that examines changes in water-quality and the abundance of Corbicula with resulting shifts in macroinvertebrate communities would be beneficial to further understand the mechanisms that cause macroinvertebrate community change, both natural and anthropogenic. A more complete understanding of the macroinvertebrate community response, based upon empirical data, would strengthen arguments for improving watershed management and protection.

INTRODUCTION

The Buffalo National River (BUFF), a unit of the U. S. National Park Service, was established in 1972 by Congress (P. L. 92-237), "for the purposes of conserving and interpreting an area containing unique scenic and scientific features, and preserving as a free-flowing stream an important segment of the Buffalo River...". Staff associated with the BUFF have conducted water-quality monitoring on the Buffalo River and its tributaries since 1985. Analyses of 10 years of water-quality data indicate that elevated nitrogen concentrations occur within the mid-reaches of the river, and that the increased concentrations are due to non-point source nutrient loading by several tributaries (Mott, 1997; Petersen, 1999 USGS draft document). Two of these tributaries, Little Buffalo River and Big Creek (upper) are quite large and also exhibit the highest nitrate concentrations. Calculated nitrogen loads (nitrate+nitrite/nitrogen) for four mid-reach tributaries (Mill Creek, Little Buffalo River, Big Creek, and Davis Creek) represent approximately 40% of the total nitrogen loading into the river, and average nitrate values are 2 - 4 times higher in these tributaries than in the adjacent river. The high concentrations of nutrients within these systems generated concern over possible detrimental effects to the biologic communities of the Buffalo National River.

The River Continuum Model (Vannote et al., 1980) suggests that "stream community structure and function adjust predictably to changes in certain geomorphic, physical, and biotic variables such as stream flow, channel morphology, detritus loading, size of particulate organic material, characteristics of autotrophic production, and thermal responses." Biological communities along this physical and energetic gradient form a spatial and temporal continuum of synchronized species replacements, which distribute energy flowing through the system in a more conserved manner. Species diversity and richness along this continuum would be expected to be highest in the region of the stream where habitat heterogeneity was most complex. Bryant (1997) hypothesized that diversity and richness would increase in a downstream manner as habitat heterogeneity increased, and that the heterogeneity in habitat complexity would continue to increase throughout his study area between the headwaters at Dixon Ford to the fifth-order reach at Highway 14. Results indicated that macroinvertebrate communities inhabiting the mid-reaches of the river deviated from the expected model. Macroinvertebrate community indices for diversity (Shannon's Index) and species richness (Margelef's Index) were lower in the mid-reaches in comparison to the headwaters and lower reaches of the river. The decline in diversity and richness in the mid-reaches of the river was attributed to increased nitrates and other associated changes linked to agriculture. The highly significant negative correlation of diversity and richness with increasing nitrate concentrations suggests a strong relationship between current land-use practices, nonpoint source runoff, and degrading macroinvertebrate communities, a result consistent with another regional study (Petersen, 1998). Other environmental factors such as physical habitat variables did not correlate strongly with richness and diversity.

The large-scale model comparison by Bryant (1997) did not provide the spatial resolution necessary to assess the magnitude of the community decline within the mid-reaches of the river. Only two of the biomonitoring stations used in the study could be considered as the mid-reaches of the river. The lack of resolution suggested two of the goals of the present study. The most important goal is the verification of the decrease in macroinvertebrate diversity and richness within the mid-reaches. With only two sites representing the mid-river, the importance of verifying Bryant's findings using a larger number of biomonitoring stations is critical in developing an argument for further watershed protection. The second goal is to determine the possible causes for the loss in diversity and richness. Associations of physical, chemical, and biological parameters with diversity and richness can be defined using correlation techniques. The strength of correlation of the variables will aid in developing a conceptual model that represents the response of the macroinvertebrate community to environmental stresses.

Corbicula (Asiatic clam) was first introduced into the United States in the Columbia River during the early 1930's as a supplemental food source for humans (Counts, 1986). Since its introduction into the Columbia River, Corbicula has extended its range to include 38 states with the northern most climates limiting the northern expansion of its range. Fox (1970) was the first to document the occurrence of Corbicula in the waterways of Arkansas. He concluded that Corbicula was already widespread throughout most stream systems. The effects of introductions of Corbicula on the biological communities are variable. Changes in native community fauna due to competition for limited food resources with Corbicula have been documented (Devick, 1991). Alterations of benthic substrates due to accumulation of shell remnant also result in faunistic changes (Sickel, 1986).

Corbicula does not produce a parasitic larval form, as do most indigenous mussels. Rather, Corbicula produces a free-swimming larval form known as a veliger. The veliger phase lasts approximately one week before metamorphosing into the bivalve which develop small byssi that allow attachment (infaunal) onto the benthic substrates

(Oesch, 1995). After a year, Corbicula is capable of reproduction as a protandric, consecutive hermaphrodite that has enormous larval production.

As a free-swimming larva, Corbicula has limited swimming ability, and dispersal of young is primarily downstream. During the adult portion of the life cycle, Corbicula is predominately sessile and lacks the capability to move large distances (Oesch, 1995). Natural dispersal is limited, but wading birds are suspected to be natural vectors. However, transportation by wading birds alone does not explain the range explosion seen in Corbicula (Counts 1986, and Isom 1986). Corbicula are used as a source of fishing bait in California and other states, which probably accounts for much of the species range expansion. Probably Corbicula disperses and colonizes isolated areas using human vectors (Counts 1986).

During Bryant's study (1997), Corbicula were discovered in several sites along the main corridor of the river. Population densities of Corbicula were considerably higher at the Woolum site (R5; 396/m^2) as compared to sites above and below (120/m^2 and 60/m^2, respectively). Large populations of Corbicula in the Buffalo River was also noted at the same location by the USGS's Ozark Highlands NAWQA researchers (personal communication – James C. Petersen). In California, densities as high as 5,000/m^2 were sufficient to cause shifts in macroinvertebrate community structure, which led to native species displacement (McCann et al., 1996), but the density of Corbicula required to displace native macroinvertebrate species is unknown for Ozarkian streams.

Successful invasions of Corbicula in aquatic communities have been linked to the level of habitat disturbance prior to their introduction. Research suggests that if the native environment is not already anthropogenically disturbed and the native faunas are not already stressed, <u>Corbicula</u> can not functionally displace native species. However, in systems where chemical and physical habitat alterations existed previous to its introduction, <u>Corbicula</u> has successfully displaced native species (Isom 1974, Fuller and Imlay 1976, Leff et al. 1990, Klippel and Parmalee 1979, Kreamer 1979, and Taylor 1980, as seen in McCann et al., 1996).

Recent evaluations of the mechanisms which form and maintain hydrologic habitats such as riffles, races, and pools in Ozark Plateau streams (McKinney, 1997) suggest that prior land-use within watersheds may induce changes into the dynamic balance of habitat formation and maintenance. One of these changes is conceptualized as a longitudinal wave of aggregates such as small-size gravel moving down the river corridor. Sources of such aggregates are attributed primarily to riparian land clearing (Jacobson and Primm, 1995). Land-use induced channel instability has probably increased the area and number of disturbed reaches in Ozarkian streams (Jacobson, 1995). Movements of sediment waves are facilitated by large flood events and are known to be more influential on a macroinvertebrate community's composition when the differences in the sediment wave particle size and the initial bed material are dramatic. When variation in velocity and depth are factored out, macroinvertebrates show distinct preferences for certain substrate sizes and diversities (Plafkin et al., 1989 and Reice, 1980). Overall, effects of changes in substrate composition within the Buffalo River on macroinvertebrate habitats are unknown. Changes in physical habitat may be one component underlying the declines of macroinvertebrate diversity and richness observed by Bryant (1997) in the mid-reaches of the Buffalo River.

OBJECTIVES

The goal for this investigation was to quantify water-quality parameters and possibly their effects on the biological resources of the Buffalo River. This study was designed to collect additional information on water-quality and community response to gain a more complete understanding of why communities appear to be deteriorating within the mid-reaches. The objectives of this ecological assessment were:

1) **Establish biomonitoring stations within the mid-reaches of the Buffalo River to compare the macroinvertebrate communities in the main channel to previously observed measures of macroinvertebrate community composition.**
2) **Examine correlations between macroinvertebrate community richness and diversity and water-quality parameters and physical habitat variables to better understand the influence of these parameters on community composition change.**
3) **Compare densities of <u>Corbicula</u> with physical habitat variables and corresponding aquatic macroinvertebrate taxa to elucidate potential relationships.**
4) **Contribute additional information to the database established by UCA regarding the macroinvertebrate communities of the Buffalo River and stream systems of the Ozark Highland Ecoregion.**

STUDY AREA

Buffalo National River is a 240-km long free-flowing stream in northern Arkansas famous for its scenic beauty as well as canoeing, fishing, and other recreational opportunities (Appendix A). Jurisdictional boundaries of the National Park Service include a continuous 211-km river corridor from near the headwaters to the confluence with the White River. Eleven percent of the watershed is within BUFF's boundaries. Remaining lands within the watershed are a mix of public and private ownership: Ozark National Forest (26%), Arkansas Game and Fish Commission (3%), and private (60%). A myriad of land-use activities, mostly related to agriculture, occur within the watershed including logging, beef, dairy, swine, and poultry operations (Mott 1997).

The Buffalo River originates in the Boston Mountains of Arkansas and flows northeasterly along the southern border of the Springfield-Salem Plateau before its confluence with the White River near Cotter, Arkansas. The topography of its watershed includes characteristics of both the Boston Mountain and Springfield-Salem Plateau physiographic regions. Sandstones and shales of Pennsylvanian age are found in the upper reaches of the river. Limestone and dolomite formations of Ordovician through Mississippian age are more prevalent in the middle and lower reaches (Mott, 1991). The rock strata in both regions are relatively horizontal and have been incised by many streams. Recent work by Mott (in press) and Hudson (in press) has demonstrated a significant degree of structural complexity within the basin and documented the major influence of Karst ground-water flow on water-quality of the Buffalo National River.

The upper watershed is located in the Boston Mountains and has a rugged terrain with steep slopes, making land largely unsuitable for agriculture; only 12% is agricultural production and 88% is forested. In contrast, the mid- and lower-reaches are more suitable for agriculture; 62% of total land area is in agricultural production and 37% is forested (ADEQ, 1991). A clear link has been established between increased pasture and increased nutrients within the Buffalo River (Mott, 1997, Petersen et al, 1998).

The total size of the Buffalo River watershed is approximately 342,196 hectares with the tributary sizes varying from the smallest monitored tributary, Ponca Creek with 1095 hectares, to the largest, Little Buffalo River at 33,117 hectares (Appendix A). Annual mean discharge (1940 to 1993) in the mid-river reaches based on USGS gauging station (Buffalo River near St. Joe, 07056000) is 29.6 cubic meter per second (cms). Mean monthly discharges range from 4.3 cms in September to 62 cms in April (USGS Report AR-93-1). For any study within the Buffalo River, hydrologic variability is a component that should be considered, and extremes in discharge can range from a historic low of 0.2 cms in 1957 to a high of 4,474 cms in December 1982 (http://ar4.darlrk.er.usgs.gov). Extremes in discharge can vary in 1 to 2 days, and provide major differences in seasonal flow patterns. Aquatic habitat formation and maintenance is more active during the high-flow regime seasons (winter and spring). Habitat formations within the Buffalo River and other Ozark streams are ultimately a product of extreme flow events, but the maintenance of these habitats is more actively defined by less extreme flows known as bank-full events (McKinney, 1997).

METHODS

Sites were selected using habitat characteristics, histories, distances relative to tributaries, and accessibility as criteria. Although no two riffle habitats are the same, efforts were made to reduce habitat variability by selecting sites that were most similar. Habitat uniformity was determined by examining the riffle geomorphology using overall shape, depth, flow pattern, and substrate size. Riffles selected for this investigation were typically single channels without meanders. Depth fluctuated slightly, but all riffles were shallow enough to exhibit surface aeration during low to moderate flows. Most riffles had a thalwag that was centrally located along the longitude axis of the riffle, although some were slightly diagonal. Substrates ranged from cobble- to cobble/gravel, and the domination of one substrate over the other was minimal. A total of 16 sites were evaluated as potential collection sites. Of the eight sites not selected, four were excluded because their substrate was largely bedrock, one was eliminated because it was too near tributary inputs, and three were relatively inaccessible (Appendix A).

Field Collections

Physical habitat evaluations were performed during low-flow conditions when channel characteristics were more easily measured. A modified first-level reach characterization (Meador et. al., 1993) was performed on all macroinvertebrate-monitoring sites. Three transects were placed within each riffle at 1/4, 1/2, and 3/4 of the length. The following parameters were measured at each transect: bankfull width, channel width, water depth, bed substrate, percent canopy cover, and embeddedness.

Depths and canopy measurements were taken at quarter lengths across each transect. Canopy densities were measured with a hand-held densiometer in four directions (north, east, south, and west) at each quartile. Substrates were measured within the channel width using a Wolman pebble count (1954). The length and width of individual substrate particles were measured using calipers and the average recorded. Substrates were selected haphazardly along each transect to lessen bias. Embeddedness was also estimated across transects by collecting cobble-size substrates and estimating the percentage that the cobble was embedded into the surrounding fines. Planview maps of the reach were drawn for each site. Maps included habitats surrounding the sample riffle and an overall view of the channel morphology.

Macroinvertebrate collections were conducted seasonally for 1 year. Spring was considered to be late March through late June, summer was late June through late September, fall was late September through late December, and winter was late December through March. Samples were collected during the peak of the season while avoiding high flow events and drought conditions (EPA, 1994).

Five Hess samples were collected from each riffle habitat during each season. Samples taken during seasons with high-flows (winter and spring) were collected using a modified Hess sampler that was tall enough (1 m) to be used at all depths. Sample locations within the habitat were haphazardly selected within the riffle habitat by blindly tossing a sample buoy marker into the riffle. Sample points were marked with a small, anchored buoy labeled with the assigned sample point number, 1 through 5. Sample 5 was the lowest positioned sample within the riffle, and sample 1 was in the upper location.

Once the sampling point was marked with the numbered buoy, physical habitat measurements and physicochemical parameters were collected from that point. Measurements of water depth (m), water velocity (m/s at 20% and 60% of depth), canopy cover (%), temperature (C°), dissolved oxygen (mg/L), pH, specific conductivity (μmhos), and turbidity (NTU) were taken from each sampling point, while being careful to minimize habitat disturbance. Once point collections of physicochemical characteristics were completed, the Hess sampler was placed into the substrate to a depth of 10 cm. Substrates from within the sampled area were washed into the net and removed for inspection of macroinvertebrates. Once completed, substrate sizes were measured using the same technique as described in the physical habitat evaluations. After a maximum of 25 substrate measurements were recorded, the remaining substrate within the sampler was mixed for 5 minutes in order to maximize the capture of macroinvertebrates. Each macroinvertebrate sample was placed into a container, labeled inside and out, and preserved with Kahle's solution (Wiggins, 1998). After sampling was completed at a given point, all sampling gear that had contact with the sample was rinsed thoroughly, examined carefully, and picked free of organisms and organic debris. Sampling equipment was also examined prior to sampling. These methods of macroinvertebrate, physicochemical, and physical habitat collection were carried out each season at each sampling site.

Water-quality samples were collected by both NPS and UCA from water-quality and macroinvertebrate monitoring stations by taking two grab samples from an area of mixing in the stream's centroid, one for nutrients and one for fecal coliform analysis. Samples were placed on ice and maintained at < 4.0 °C throughout their transportation and shipment. Holding times for nutrients did not exceed 48 hours, and fecal coliform analysis did not exceed 4 hours (Table 1, Hach 1987). Physicochemical measurements also were made in the stream's centroid, and the following parameters were measured: pH, dissolved oxygen (mg/L), specific conductivity (μmhos), temperature (^{o}C), and turbidity (NTU). Twelve sampling runs were attempted by UCA on all sites, and NPS collected samples quarterly at Pruitt (R3) and Woolum (R5) with analysis conducted by ADEQ. Water-quality samples collected by NPS began two months prior to the first collection of macroinvertebrate community data (4/9/97), and sampling continued on a quarterly basis until two months past the last macroinvertebrate sampling event (7/6/98).

Laboratory Analysis

Macroinvertebrate samples were placed into a white enamel pan, and all organisms were hand-picked from the organics and placed into groups at the ordinal level. All organisms were identified to the lowest taxonomic level practical (genus in most cases), and counted. Taxonomic keys used included Merritt and Cummins (1996), Stewart and Stark (1993), Wiggins (1998), Oesch (1995), Bednarik and McCafferty (1979), Hobbs (1976), Kondratieff and Voshell Jr. (1984), Pennak (1989), Pflieger (1994), and Provonsha (1990). Data were recorded on laboratory taxonomic forms and entered into a computer.

Taxonomic quality assurance/ quality control (QA/QC) was maintained by constructing a reference collection of taxa encountered throughout the study (EPA, 1994). Specimens were sent to a regional macroinvertebrate taxonomic specialist for validation. A species collection logbook was kept to record general and site specific information on each individual referenced in collection. Notes on sample condition and taxonomic anomalies were noted on laboratory taxonomic forms and comments were included in the electronic data set.

Water-quality samples were returned to UCA and placed individually into clean beakers and allowed to warm to 23°C. Once sufficiently warmed, the samples were filtered through a 0.45-micron fiber filter in order to remove suspended materials. The samples were analyzed for nitrate/nitrogen (0.005 to 0.40mg/L, NO_3^-), nitrite (0.005 to 0.300mg/L, NO_2^-), and reactive phosphorous (0.005 to 2.50mg/L, PO_4) (Table 1). Procedures for the analysis of both compounds are outlined in the Procedures for Water and Wastewater Analysis (Hach, 1987). Throughout the analyses, ambient room temperatures were monitored for change, and room temperatures recorded in the water-quality notebook. Spectrophotometer readings of transmission and absorption were recorded in the water-quality laboratory handbook. A standard curve for each nutrient was constructed, and results were recorded in concentrations of mg/L. Procedures for cleaning glassware used in filtering and analysis between sites were as follows: wash with 3% phosphate free soap, rinse three times with tap water, and rinse three times with deionized water. Cleaning procedures for glassware between collections trips were as follows: rinse three times with tap water, wash in 3% phosphate free soap, rinse three times with tap water, soak in 5% HCl acid for 15 minutes, rinse three times with deionized water, and then store in a dust free environment.

Table 1. Water-quality parameters collected by UCA with holding times, detection limits and error limits.

Parameters	Preservation	Holding Times	Detection Limit	Error Limit
pH	Field measurement	NA	NA	+/- 0.05
Specific Conductance (uS/cm)	Field measurement	NA	NA	+/- 3.0
Dissolved Oxygen (mg/L)	Field measurement	NA	NA	+/- 0.02
Turbidity (NTU)	Field measurement	NA	NA	+/- 0.5
Temperature (°C)	Field measurement	NA	NA	0.1°C
Nitrate (NO_3^-)	Ice <4.0°C	48 hours	0.07mg/L	0.001mg/L
Nitrite (NO_2^-)	Ice <4.0°C	48 hours	0.04mg/L	0.001mg/L
Reactive Phosphorous (PO_4^{3-})	Ice <4.0°C	48 hours	0.006mg/L	0.003mg/L
Fecal coliform (Colonies/100ml)	Ice <4.0°C	4 hours	1/100ml	NA

Quality Assurance/ Quality Control (QA/QC) was maintained by sampling 1 QA/QC sampler per 10 environmental samples (Appendix B). Trip blanks, duplicates, replicates, and equipment blanks were used to assess possible contamination of sampling, processing, and analysis procedures. All findings and comments on procedures and results were recorded into a bound water-quality lab notebook.

Fecal coliform analysis was performed in both field and laboratory conditions. In field conditions, a Millipore® filtration kit was used to process fecal coliform samples. Sterilization of filter apparatus was accomplished by combustion of a methanol flame, creating formalin gas, which kills adhering bacteria. Post-sterilazation rinsing and sample dilutions were made using a standard phosphate buffer solution (Shelton, 1994). Filtered samples were placed into petri dishes with absorbent pads containing 2 ml of MF-C media. Samples were incubated at 44.5°C for 18 to 24 hours. A mobile field incubator was used for samples processed in the field. Lab samples were handled in the same manner, but instead of the filtration kit, a multiple sample vacuum system and glass funnel filters were used for laboratory filtrations. Glassware was sterilized using an UV light sterilizer with an exposure time of 5 minutes. One QA/QC blank sample per 10 environmental samples was run in order to assess possible analysis contamination.

Data Analysis

Shannon's index of diversity was generated for 155 samples collected during the four seasons. The Shannon index equation used was the same as in Bryant (1997), and the equation was $H' = (N \log N - \Sigma[n_i \log n])/N$. The letter "N" represented the total abundance of sample, and "n" represented the abundance of a particular taxon. Shannon's index was selected over other diversity indices because of the previous use in Bryant (1997) and because it incorporates a component of community evenness into the equation.

Databases were constructed for the physical habitat data, macroinvertebrate data, and water-quality data in an Excel worksheet format (Microsoft Excel 97, SR-1). Pearson's product moment correlation coefficients were used in a matrix format in order to provide "lines of evidence" into possible associations of community characteristics with physical habitat variables and water-quality values. When the majority of variables did not conform to the normality assumption of Pearson's correlation, and log transformations were unsuccessful or the sample size was small (i.e. riffles), a Spearman's rank correlation was used. Rank correlation, such as Spearman's, does not require assumption of normality on degree of determination between random variables, and can demonstrate stronger attributes of the measurement of relationships that are not linear in nature (Johnson et al., 1997).

When Pearson's product moment correlation coefficients were generated, a Bonferroni's probability matrix was also generated in order to determine statistical significance of the variables. Bonferroni reduces the systematic inflation of alpha, and produces a weighted probability value for the number of correlations made in the matrix. Systat®, the statistical package used in the analysis, performs this function automatically for each matrix selected, and acceptance of significance was based upon an adjusted probability value of 0.05.

Multiple Regression (Model II) was used in weighing the dependence of Corbicula on the physical habitat variables. The dependent variable was the density of Corbicula. Physical habitat variables were considered independent variables. Assumption tests for regression and correlation are similar (Berk, 1994; Sokal and Rohlf, 1995); therefore, the assumption test described in the correlation section will also apply to the regression model. Once qualifying data tests were conducted, the results of the multiple regression analysis were examined for the strength of squared multiple (R^2) and the tolerance values listed in the output for each independent variable. Tolerance values generated in regression models for individual predictors (independent variables) are the proportion of the variability that explains the relationship to the dependent variable (Berk, 1994). Therefore, tolerance values were used to exclude low value predictors based upon their redundancy in the analysis. During the exclusion of low predicting independent variables, the R^2 was monitored for the overall strength of the association of the dependent variables and its predictors. The combination of independent variables that resulted in the strongest R^2 values was accepted as the final model and the contributions of the independent variables were discussed.

Data variables from five data sets were tested for the assumptions required by Pearson's product moment correlation test. The five data sets were 1) physical habitat data, 2) NPS and ADEQ water-quality data, 3) UCA water-quality data, 4) macroinvertebrate community assessment data, and 5) the combination data set which included the averaged macroinvertebrate community indices and averaged UCA water-quality data. The macroinvertebrate community assessment data were structured into three levels of scale. The riffle habitat scale had a sample size of 5, the seasonal scale had a sample size of 35 and 40, and the largest scale, the year scale, had a sample size of 155. At the riffle habitat scale, Spearman's rank correlation was used in place of Pearson's because low sample sizes are seldom normal in distributions. The physical habitat data were collected from 3 transects within the 8 riffle habitats, and therefore had a sample size of 24. The following variables were measured and tested for Pearson assumptions: bankfull width, stream width, riffle length, canopy, mid-depth, average substrate size, and percent embeddedness. Five of the seven variables could not be log transformed into a normal distribution. Therefore, Spearman's rank correlation coefficient values were used to test association strengths.

RESULTS

Physical Habitat

Six sites ranged between 35 to 95 m in bankfull width (Figure 1). Of the two that were outside the range grouping, site R4.1 was the narrowest (29.1 m to 31.3 m) and site R5 was the widest (180 m to 186 m; see Table 3). Variation in measurements within riffle habitat at each site was largest at site R3 (35 m to 54.4 m) and smallest at site R4.1 (29.1 m to 31.3 m). Site R5 was approximately 3 times as wide in bankfull width as the other sites.

Stream widths, with an exception of three sites, were less than 30 m in width (Figure 2). Sites R3.2, R4.2, and R4.3 were notably above the 30 m and were all associated with well-developed lateral gravel bars. Site R4.2, below the confluence of Big Creek, was the widest ranging from 80 to 90 m wide. R3.2 and R4.2 riffle habitats terminated into lateral bluff pools that had strong lateral shift of channel at the head of the bluff pool habitat, thus propagating the upstream lateral gravel bar development and increasing stream width.

Canopy densities were the highest at R3, R4.1, and R3.1 (Figure 3). R3 had the largest variation in canopy cover, ranging from 8 to 53 percent coverage, with the downstream end of the habitat being most covered (Table 3). R4.1 ranged from 18 to 48 percent coverage, and R3.1 ranged from about 8 to 28 precent. All other sites were below 5 percent canopy coverage and were considered to be completely open to sunlight.

Transect mid-depths were measured at the center of the cross-section. All measurements were under 0.35 m, with the average depth approximately 0.18 m. The site was shallowest and had the widest range in depth was R4.3, which ranged from 0.04 to 0.24 m. The deepest site was R3 at 0.34 m (Table 3).

Average substrate size was similar throughout the sites except for R4.1, R4.2, and R4.3. Sites R4.1 and R4.3 had the smallest average substrate sizes. Site R4.1 had the lowest average, 57 mm. Site R4.2 exhibited the highest average size, which had an average of 92 mm (Figure 4). Sites R4.1 and R4.2 were located upstream and downstream from Big Creek, respectively, with a distance of approximately 3.2 kilometers separating these sites, which exhibited the widest difference in average substrate size. R4.2 is 1.3 km below Big Creek. The percent of dominant substrates measure also shows that sites R4.1 and R4.3 are lowest in average substrate size, and are near equal in the percentage of cobble versus gravel composition (Figure 5).

Percent embeddedness for all sampling sites ranged from 10 to 34.6%, with the highest R4.3 and the lowest R3.2. Embeddedness, a measurement of the fines surrounding cobble substrates, was highest at sites where the percent gravel was near 50% of the substrate composition (Figures 5 and 6). Embeddedness also tended to be high at sites with lower average substrate sizes, R4.1 and R4.3 (Figure 4 and 5). Comparison of the three substrate measurements shows that sites R4.1 and R4.3 are nearly equal in gravel/cobble composition, a condition not shared with other sites.

Strengths of associations were tested between bankfull width, stream width, length, % canopy, mid-depth, average substrate size, and % embeddedness. Since many of the variables were non-normal in their distributions, a Spearman's rank (r_s) correlation matrix was used to test the strength of association. The strongest coefficient resulting from the analysis was that of stream width and % canopy ($r_s = -0.768$, Table 2). Stream width was associated with mid-depth (r_s = -0.525). Other lesser associations were found with bankfull width and stream width (r_s = 0.489), bankfull width and percent embeddedness (r_s = -0.477), and bankfull width and canopy (r_s = -0.463) (Table 2).

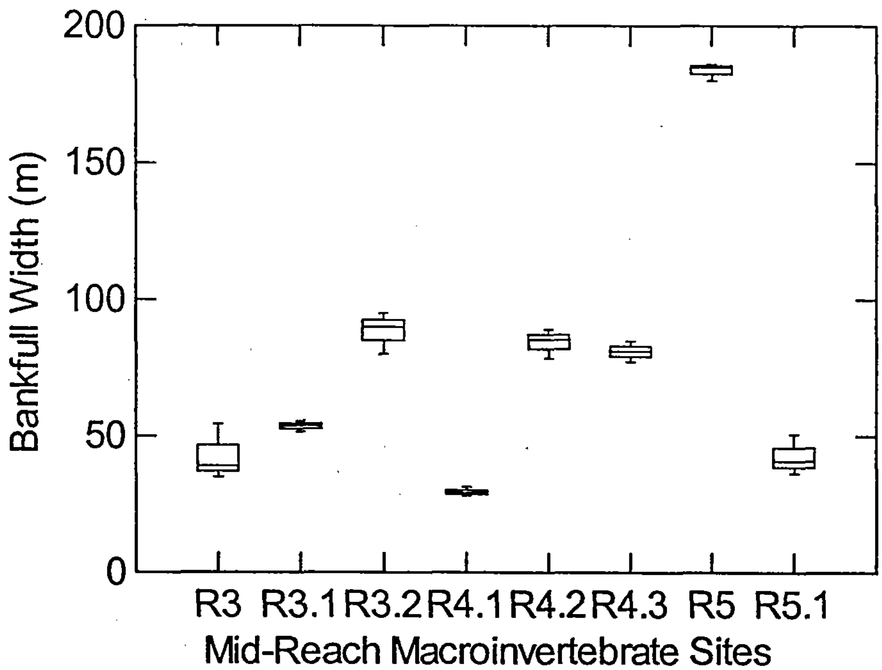

Figure 1. Site comparisons of Bankfull width from transect data on the mid-reach macroinvertebrate study sites.

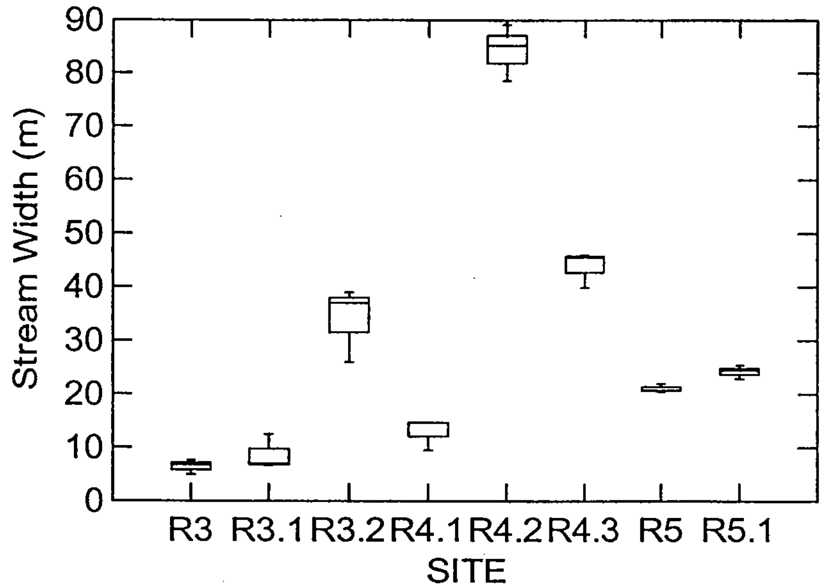

Figure 2. Site comparisons of stream width from transect data on the mid-reach macroinvertebrate study sites.

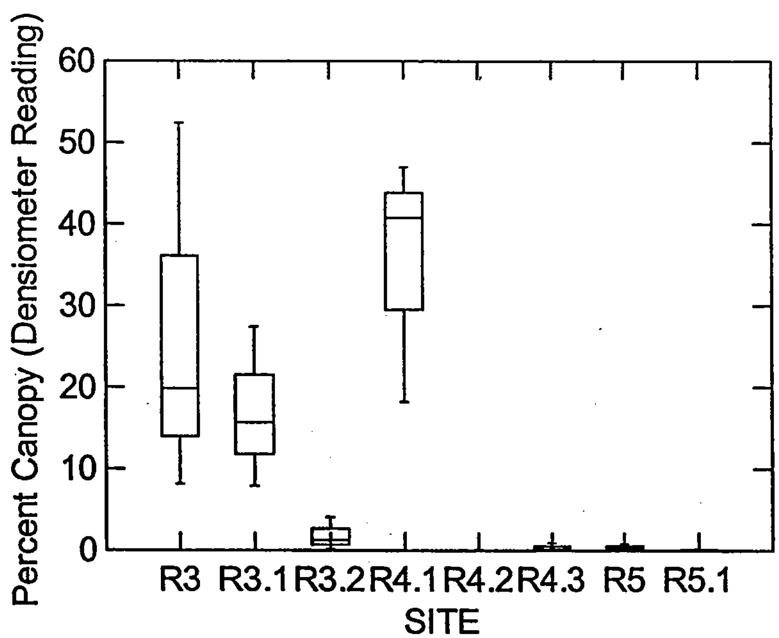

Figure 3. Site comparisons of percent canopy from transect data on the mid-reach macroinvertebrate study sites.

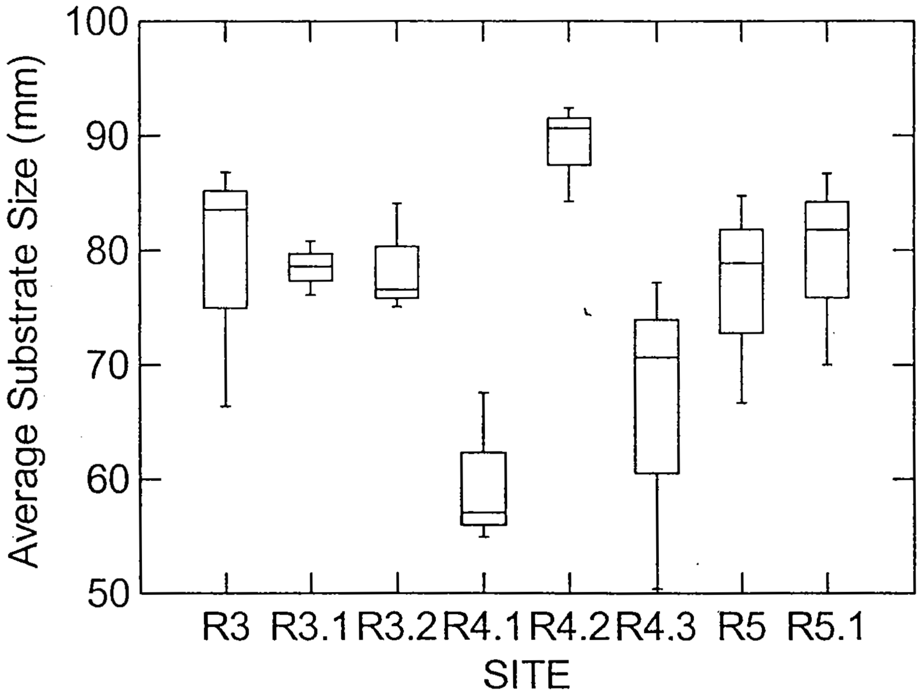

Figure 4. Site comparisons of average substrate size from transect data on the mid-reach macroinvertebrate study sites.

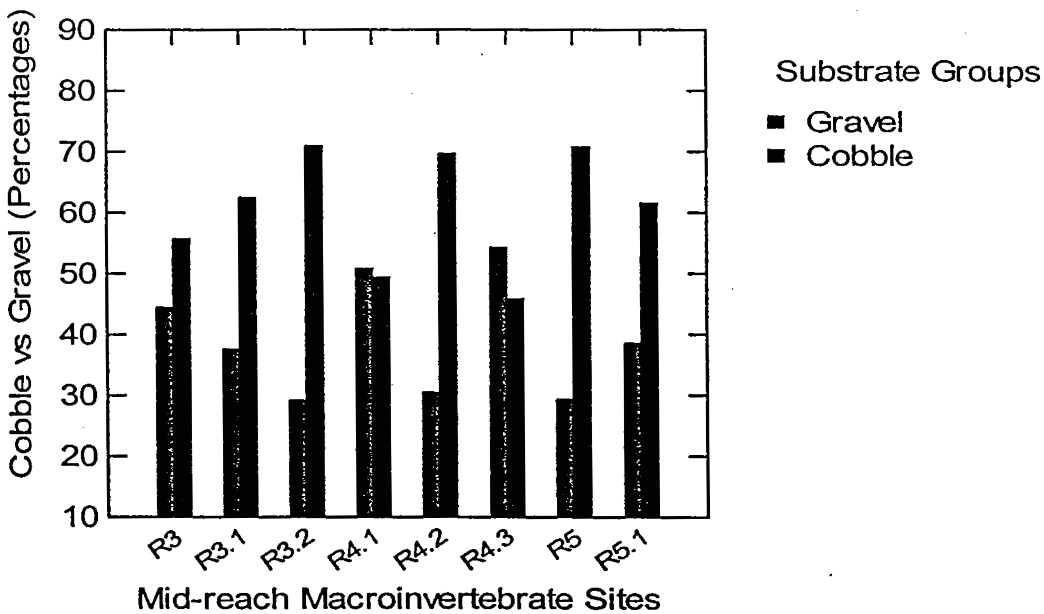

Figure 5. Percent substrate type for transect data from the mid-reaches of the Buffalo River.

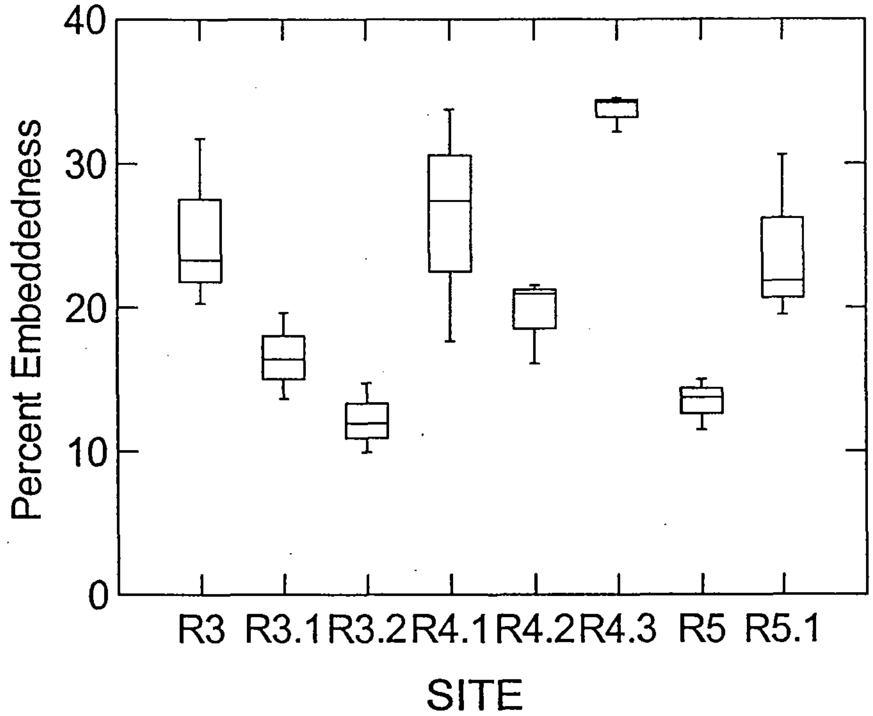

Figure 6. Site comparisons of Percent Embeddedness from transect data on the mid-reach macroinvertebrate study sites.

Table 2. Spearman's rank correlation coefficient (r_s) matrix for physical habitat variables measured from transects in mid-reach study sites.

Variables	Bankfull	Width	Length	Canopy	Mid-Depth	Substrate	Embeddedness
Bankfull Width	1.000						
Stream Width	0.489*	1.000					
Riffle Length	-0.031	0.209	1.00				
Canopy	-0.463*	-0.768*	-0.414	1.00			
Mid-Depth	0.005	-0.525*	-0.345	0.294	1.000		
Substrate Size	0.242	0.253	0.116	-0.313	-0.050	1.000	
Embeddedness	-0.477*	0.045	0.459*	-0.075	-0.057	-0.231*	1.000

* Denotes coefficient values discussed in text.

Table 3. Physical habitat variables by transect as measured from macroinvertebrate sampling sites within the mid-reaches of the Buffalo National River.

Sampling Sites	TRANSECTS	Bankfull Width (M)	Width (M)	Length (M)	Canopy (%Coverage)	Mid-Depth (M)	Percent Gravel	Percent Cobble	Substrate Averages (mm)	Substrate Counts (n value)	Percent Embeddedness
R3	1	54.4	5	59	19.8	0.31	53.8	46.2	66.4	52	23.3
R3	2	39	7.6	59	8.2	0.32	41.7	58.3	86.8	48	31.8
R3	3	35	6.7	59	52.4	0.17	37.7	62.3	83.5	53	20.3
R3.1	1	51.5	12.5	66	15.7	0.17	48.8	51.2	76.1	41	16.4
R3.1	2	53.7	7	66	27.4	0.19	33.3	66.7	78.6	48	13.7
R3.1	3	55.5	6.6	66	7.9	0.27	30.6	69.4	80.8	36	19.7
R3.2	1	80	39	46	4.1	0.11	30.5	69.5	76.6	59	12.0
R3.2	2	90	37	46	1.3	0.2	24.0	76.0	84.1	50	10.0
R3.2	3	95	26	46	0.2	0.18	32.9	67.1	75.1	79	14.8
R4.1	1	31.3	14.7	46	18.3	0.17	41.4	58.6	67.6	58	27.4
R4.1	2	28	14.7	46	40.8	0.22	56.0	44.0	57.1	50	17.7
R4.1	3	29.1	9.5	46	47.0	0.26	54.8	45.2	54.9	42	33.8
R4.2	1	78.5	78.5	64.5	0.0	0.18	39.2	60.8	84.3	51	21.6
R4.2	2	89.1	89.1	64.5	0.0	0.21	27.9	72.1	90.7	43	21.0
R4.2	3	85.2	85.2	64.5	0.1	0.1	24.1	75.9	92.4	54	16.1
R4.3	1	84.8	46	66.5	0.9	0.04	41.7	58.3	77.2	48	32.2
R4.3	2	81.1	45.6	66.5	0.2	0.04	40.4	59.6	70.6	47	34.6
R4.3	3	77.2	39.9	66.5	0.0	0.21	80.5	19.5	50.4	41	34.3
R5	1	180	20.5	50	0.3	0.27	25.0	75.0	84.8	40	13.8
R5	2	185	22	50	0.3	0.19	27.8	72.2	78.9	36	11.5
R5	3	186	20.8	50	0.8	0.23	35.1	64.9	66.7	37	15.0
R5.1	1	50.6	24.4	66	0.0	0.09	45.5	54.5	70.0	55	19.5
R5.1	2	40.8	22.9	66	0.0	0.18	32.7	67.3	86.7	55	21.9
R5.1	3	36.4	25.4	66	0.2	0.14	37.3	62.7	81.8	51	30.6

NPS and ADEQ Water-Quality

A hydrograph for the full period of study was generated using U. S. Geological Survey (USGS) data from the Highway 65 gauging station (USGS, ID07056000, and Figure 7). Extremes in the hydrograph for the duration of study were 420 cms (14,800 cfs) and 1.3 cms (5.8 cfs). During the critical low-flow period (late summer and early fall) the river experienced intermittent drying just below R5 which extended down river for 6.4 kilometers, and reemerged at the Margaret White Spring in the center of the river's channel.

Differences in mean discharges from river stations were orders of magnitude larger than discharge means observed from adjacent tributaries (Figure 8). Within the mid-reaches, Davis Creek had the smallest hydrologic contribution with Cave Creek, Mill Creek, Big Creek, Little Buffalo River, and Richland Creek contributing with increasing magnitudes. During the summer and fall, Cave Creek had extremely low flows, and Richland Creek went dry from the mouth to approximately 7 km upstream.

Mean nitrate concentrations along the river's corridor (Figure 9) give an indication of the degree of influence that tributaries have on mean nitrate (NO_3) levels within the river (Appendix C). An increase in mean concentration was observed between R3 and R4, and contributing tributaries were Mill Creek (T4) and the Little Buffalo River (T5). However, between Hasty (R4) and Woolum (R5), a slight decrease in mean concentration was observed. Within the declining reaches, Big Creek, Davis Creek, and Cave Creek were the contributors.

An individual sample taken from Mill Creek during fall was recorded at 0.725 mg/L with a discharge of 18.34 m^3/s (Appendix C). This concentration was 4.8 times higher than the concentration observed at R4 (0.023 mg/L at 83.0 m^3/s; Appendix C). Mill Creek's load calculation indicates that 389.5 mg of nitrate per second was entering the Buffalo River at the time of collection. The distance between the confluence of Mill Creek and the river station at Hasty (R4) is approximately 11.3 kilometers, with the Little Buffalo River 5.7 km below Mill Creek. Davis Creek (T7) was the next highest contributor of nitrates with a mean of 0.453 mg/L, but was the smallest in mean discharge (Figure 9).

Orthophosphate concentrations were similar among the mid-reach sites (Figure 10). No upward or downward trend was observed within the mid-reaches. However, an increase above the study area was observed. Davis Creek was highest in mean concentrations for orthophosphates among the tributaries (0.035 mg/L, Appendix C). Mid-reach tributaries were not notably higher than other tributaries above or below the study area; however, Davis Creek exhibited the highest mean throughout all the tributaries within the water-quality network. Within the mid-reaches, Davis Creek was highest; Cave Creek, Little Buffalo River, Richland Creek, Big Creek, and Mill Creek followed in descending order.

Densities of fecal coliform bacteria were recorded as the number of colonies per 100 ml (Figure 11). The highest fecal coliform level within the river was at Ponca (R2), and densities declined throughout the three mid-reach stations. Another peak was observed at Highway 14 (R7), a station well below the study area. Mid-reach tributaries were not notably higher than tributaries above and below the study area. Of the study tributaries, Mill Creek was highest, then Davis Creek, Little Buffalo River and/or Richland Creek, Big Creek, and Cave Creek followed in descending order.

Associations were examined by Pearson's correlation for water-quality parameters for all sites and all seasons. Fecal coliform was found to be associated with orthophosphates (r = 0.312; Bonferroni's 0.013). Nitrates (NO_3) and orthophosphates (PO_4) generated a significant relationship (r = 0.435; Bonferroni's 0.010). Figures 12 and 13 present the relationships between the significant variables with regression lines and confidence interval lines.

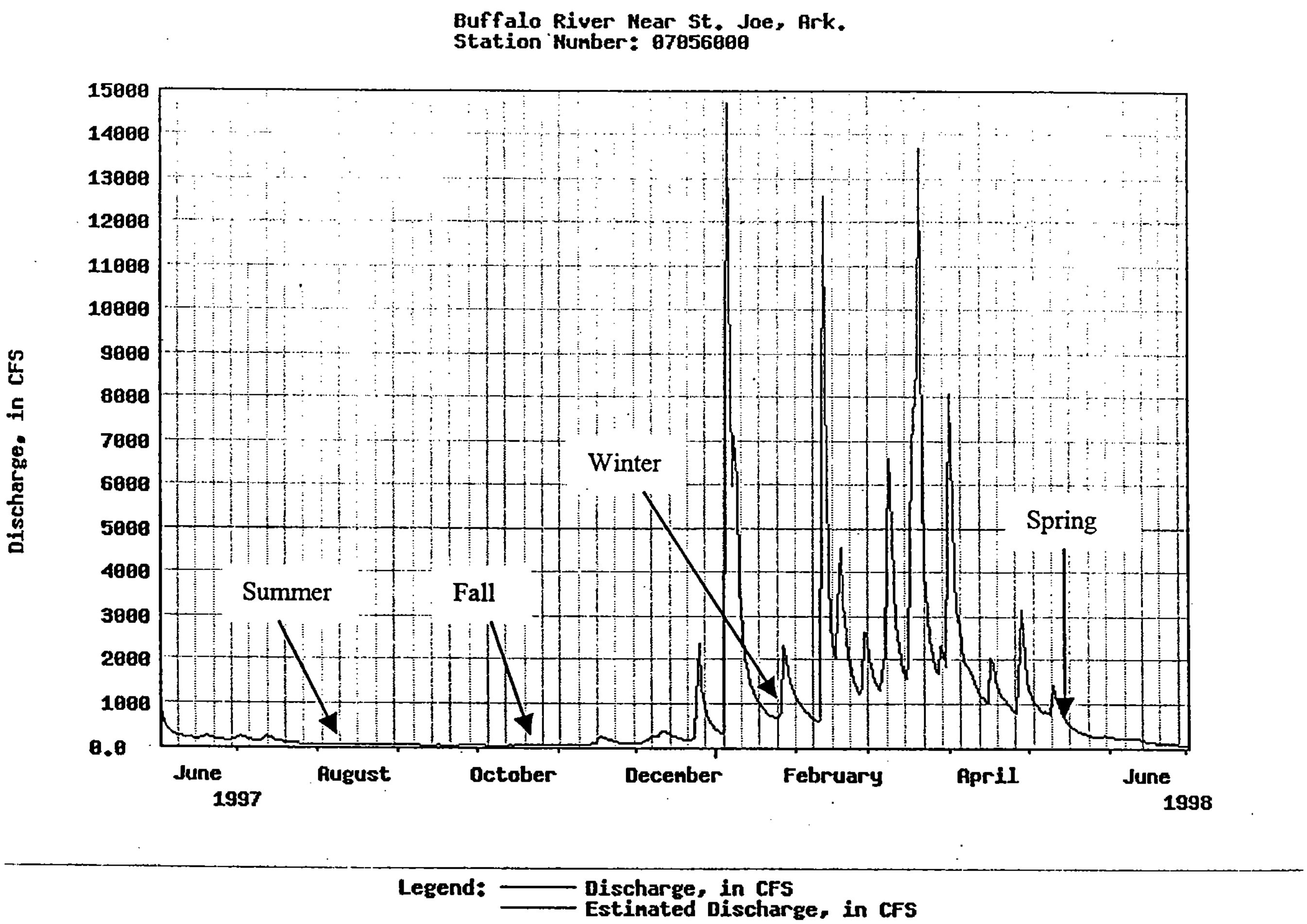

Figure 7. Hydrograph from station 07056000, Buffalo River near St. Joe, Arkansas. Discharge in cubic feet per second with points of macroinvertebrate sampling periods highlighted. (U. S. Geological Survey: http://arkweb.er.usgs.gov).

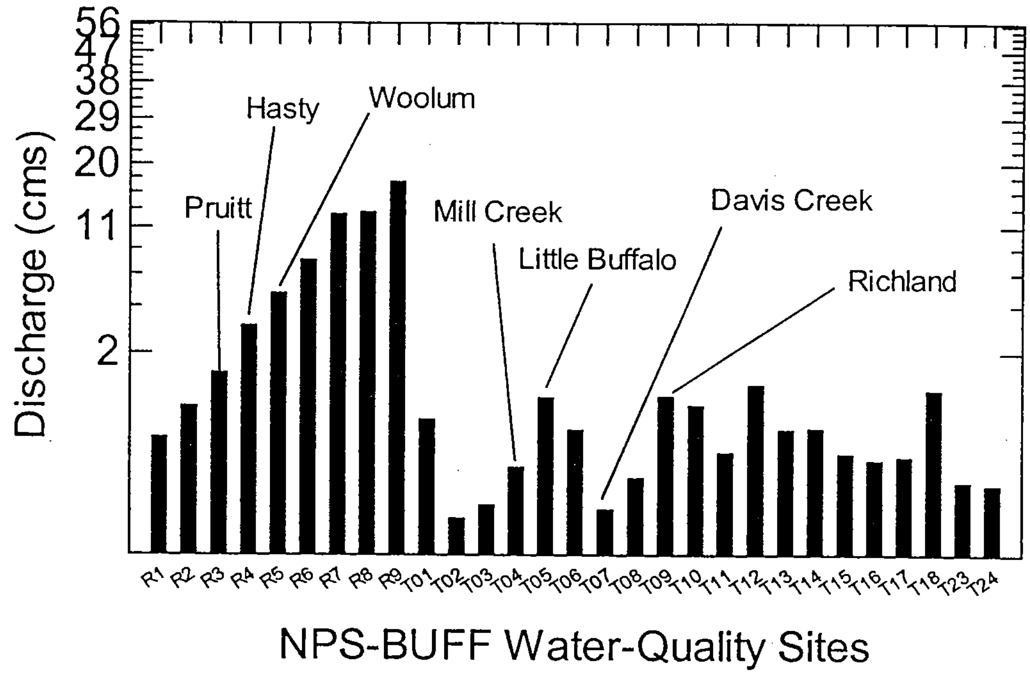

Figure 8. Mean discharges (cubic meters per second) for all NPS river (R1-R9) and tributary (T01-24) water-quality sites. Means represent the discharges from April 1997 to July 1998.

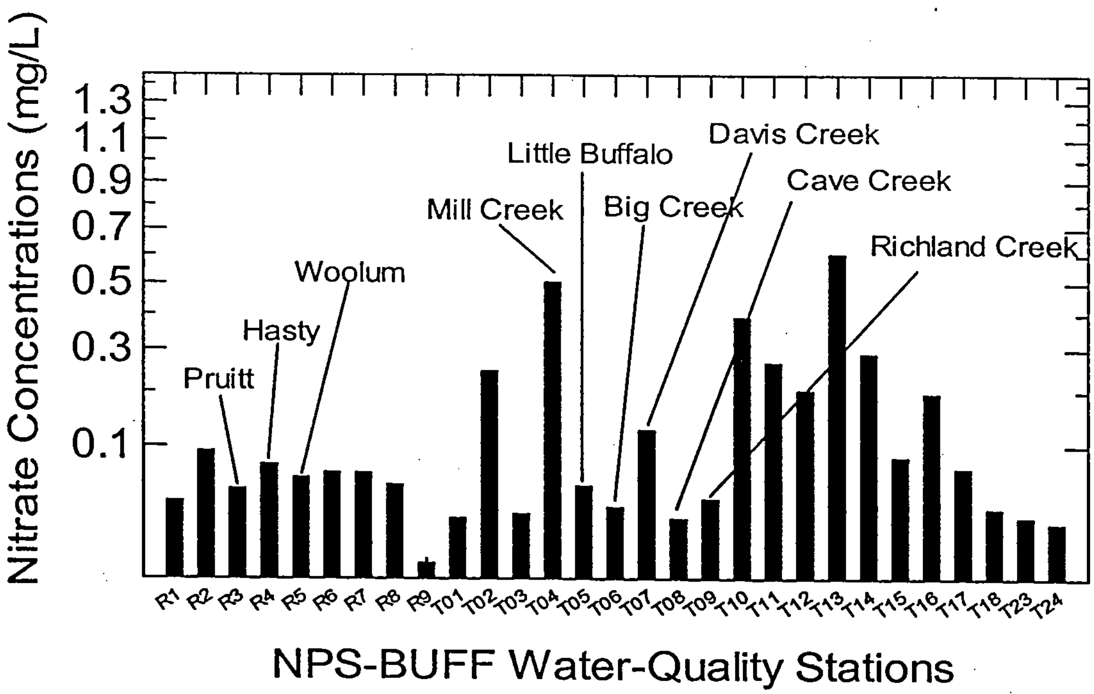

Figure 9. Mean nitrates (NO_3) concentrations from river (R1-R9) and tributary (T01-T24) sites within the Buffalo National River's Water-quality Monitoring Network. Means represent nitrate concentrations from sites during the period of April 1997 to July 1998.

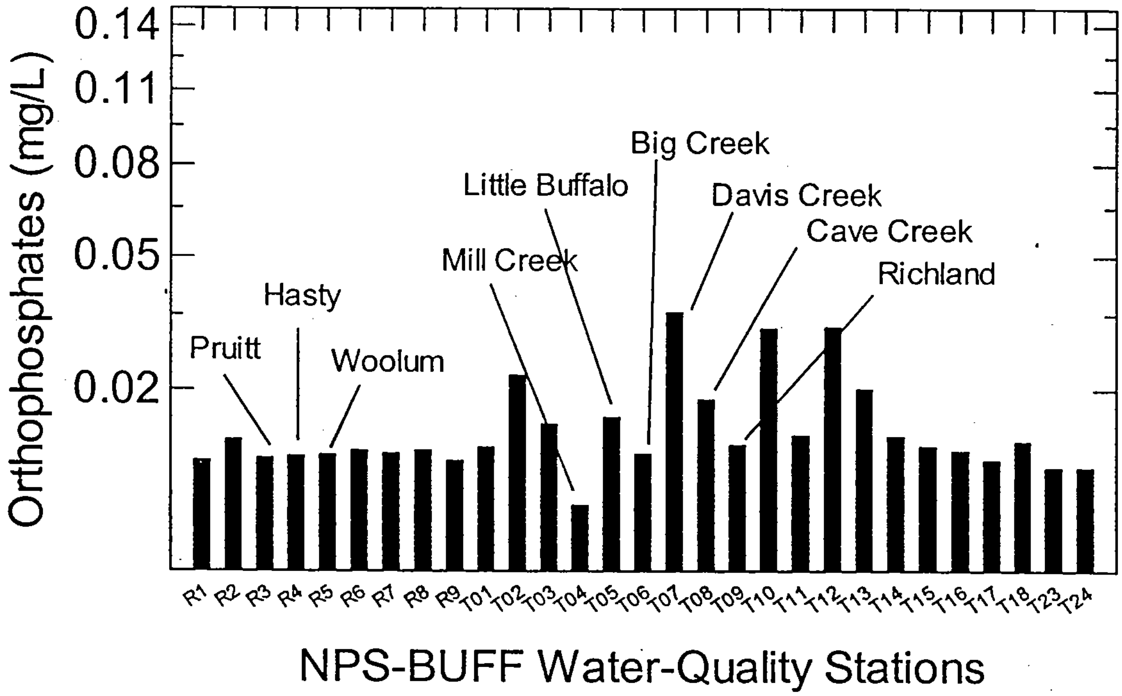

Figure 10. Mean orthophosphate (PO_4) concentrations from river and tributary sites within the Buffalo National River's Water-quality Monitoring Network. Means represent nitrate concentrations from sites during the period of April 1997 to July 1998.

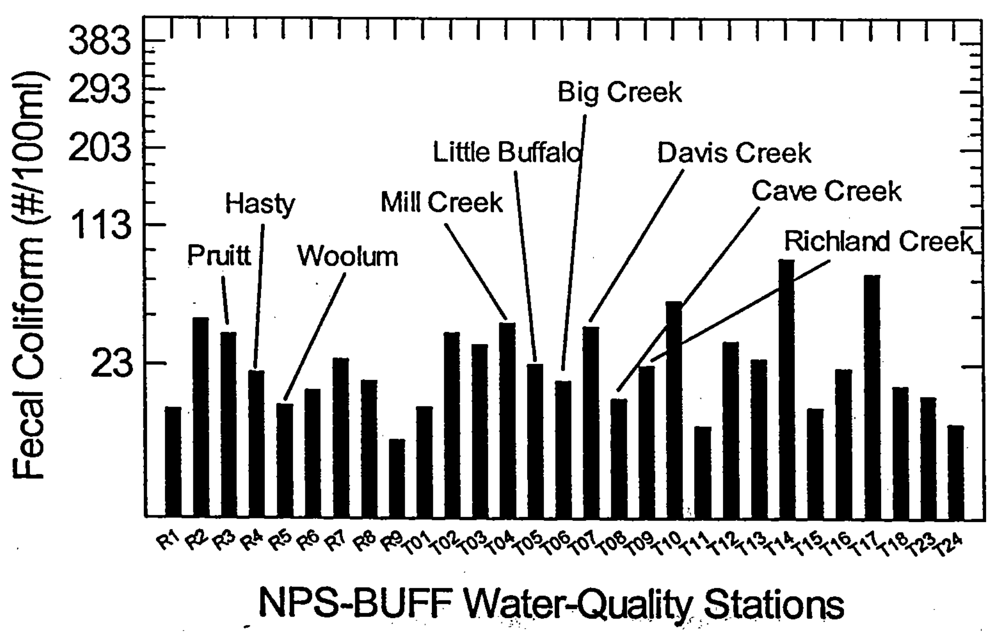

Figure 11. Mean fecal coliform densities from river and tributary sites within the Buffalo National River's Water-quality Monitoring Network. Means represent nitrate concentrations from sites during the period of April 1997 to July 1998.

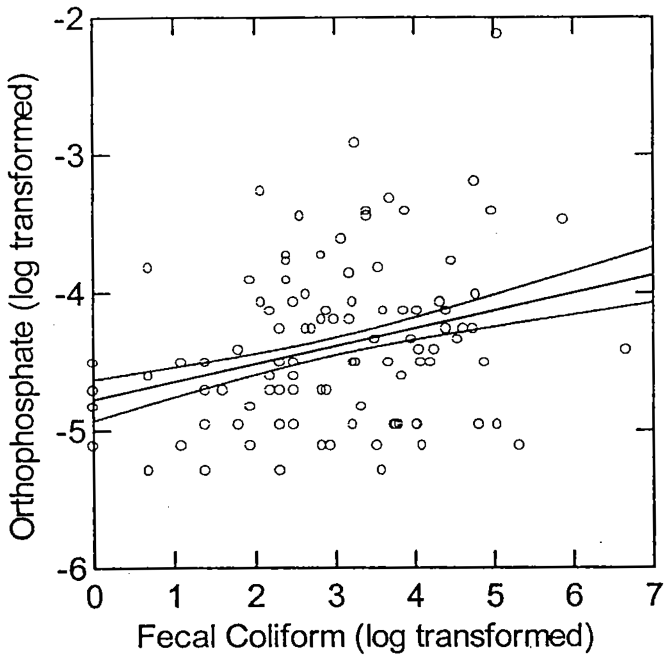

Figure 12. Scatterplot of orthophosphate and fecal coliform density (log transformed) with regression line and confidence interval brackets. Correlation based on data from NPS-BUFF water-quality sites for all seasons.

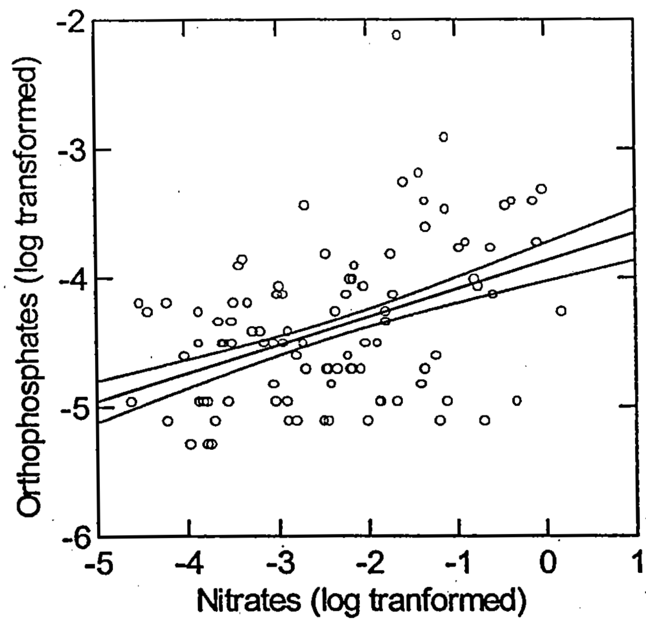

Figure 13. Scatterplot of orthophosphate and nitrate concentrations (log transformed) with regression line and confidence interval brackets. Correlation based on data from NPS-BUFF water-quality sites for all seasons.

University of Central Arkansas' Water-quality Collections

Mean nitrate (NO_3) concentrations increased downstream before peaking at site R4.3 located below Davis Creek and Cave Creek (Figure 14 and Appendix D). The most dramatic increase in nitrates occurred below Mill Creek with an approximate 44% increase in mean nitrate concentrations (Table 4). Increases in mid-reach nitrate concentrations were observed below Mill, Big, Davis, and Cave Creeks. Although, hydrologically, these tributary inputs were small in magnitude to the Buffalo River (Figure 8), their concentrations were ample to effect the river's nitrate level. A decrease in nitrate concentrations between R4.3 and R5 was observed; no permanent flowing tributaries were located within this river reach. The increase in nitrate concentration between R3.2 and R4.1 indicate undefined inputs in the Hasty (R4) area that are contributing noticeable nitrate loads.

Mean orthophosphate concentrations (PO_4) along the mid-reach study area was highest at R3.1, below Mill Creek. The next largest increase was downstream from Big Creek, and then below Davis and Cave Creeks. The lowest concentration of orthophosphates was at R5.1, the site below Richland Creek. A decrease in mean orthophosphate concentration was noted between R4.3 and R5, and was coincident with the nitrate declines for this river reach.

Mid-reach fecal coliform concentrations were highest at R3.1, just below Mill Creek (Figure 16). The second highest concentration was at R4.2, below Big Creek. Decreases in fecal coliform densities occurred at R3.2 (below Little Buffalo River), R4.1 (below the Hasty area), R4.3 (below Davis and Cave Creeks), and a slight decrease at R5. The river reach between R4.3 and R5 dropped slightly in fecal coliforms, and the decrease was coincident with decreases in nitrates and orthophosphates. Pruitt (R3) was a control site for the study, the best site for water-quality and macroinvertebrate community values, and it was the third highest in fecal coliform densities.

A Pearson correlation performed upon UCA water-quality parameters yielded only one significant coefficient (Figure 17). A positive correlation was found between orthophosphates and nitrate concentrations (r = 0.485; Bonferroni's 0.002). No relationship between orthophosphates concentrations and fecal coliform densities were found with this data set, as was seen previously with the NPS data.

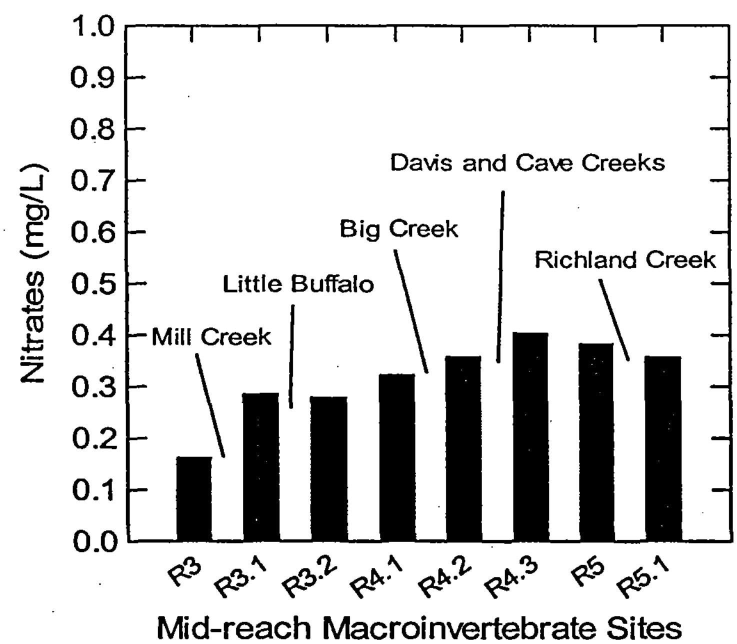

Figure 14. Mean nitrate concentrations (NO_3) from the macroinvertebrate sampling sites based upon UCA collections. Tributary designations represent their inputs into the river system as it relates to water-quality sampling stations.

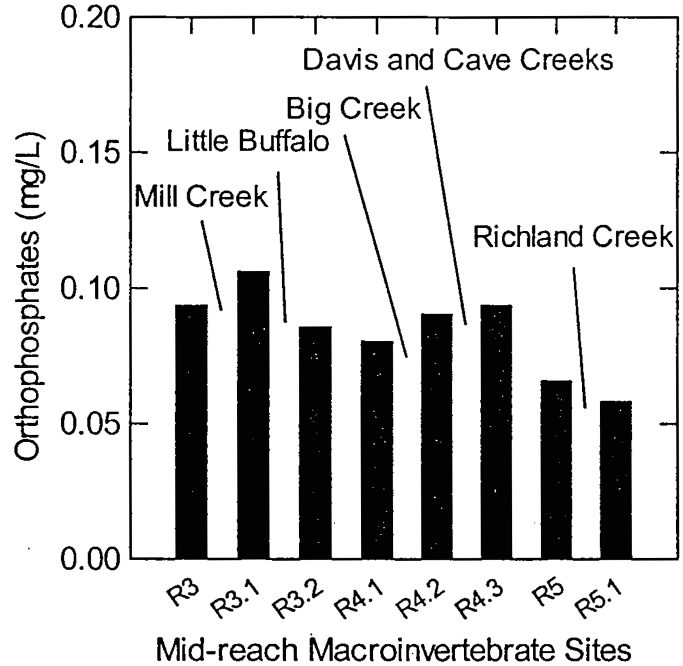

Figure 15. Mean orthophosphate concentrations (PO_4) from the macroinvertebrate sampling sites based upon UCA collections. Tributary designations represent their inputs into the river system as it relates to water-quality sampling stations.

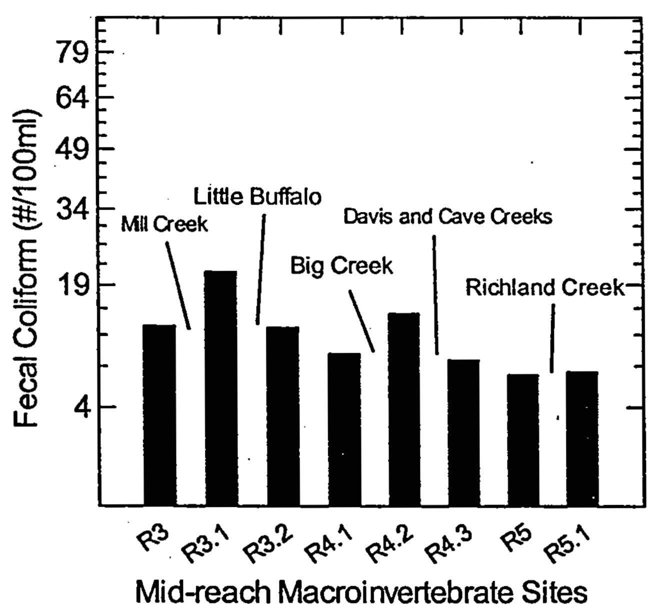

Figure 16. Mean fecal coliform densities from the macroinvertebrate sampling sites based upon UCA collections. Tributary designations represent their inputs into the river system as it relates to water-quality sampling stations.

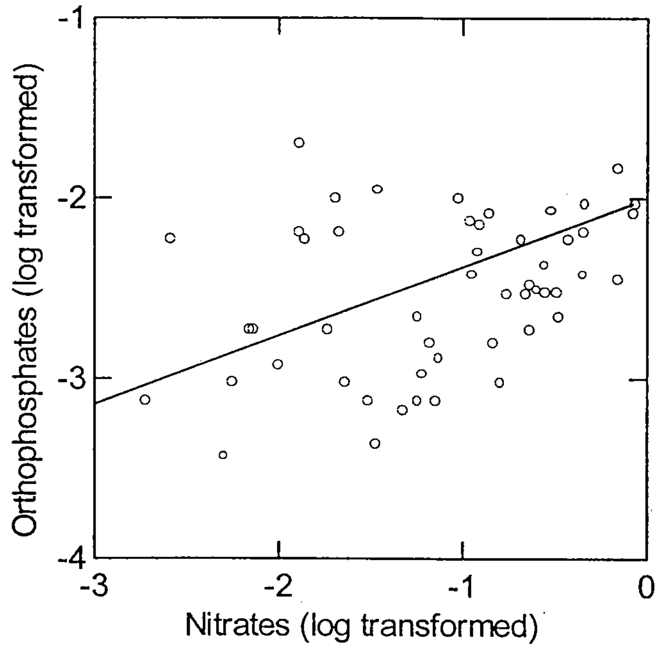

Figure 17. Scatterplot with regression line for orthophosphates (PO_4) and nitrates (NO_3) based upon UCA water-quality collections.

Table 4. Mean water-quality values for UCA data collected from November 1997 to April 1998.

Sites	Fecal Coliform	pH	Specific Conductivity	Turbidity (NTU)	Dissolved Oxygen (mg/L)	Temp (^{0}C)	Discharge (m3/s)	Nitrates (mg/L)	Phosphates (mg/L)
R3	36	7.8	130	7.7	11.2	7.4	48.8	0.16	0.094
R3.1	52	8.0	153	8.7	11.4	7.6	54.9	0.29	0.106
R3.2	15	8.2	160	2.9	11.3	8.4	29.0	0.28	0.086
R4.1	13	8.1	190	3.0	11.2	8.1	31.2	0.32	0.080
R4.2	20	8.1	201	3.3	11.2	8.7	39.4	0.36	0.090
R4.3	14	8.1	197	6.4	11.1	8.9	40.8	0.40	0.094
R5	13	8.1	186	4	11.4	8.8	37.9	0.38	0.066
R5.1	15	8.0	172	4	11.3	8.9	50.1	0.36	0.058

Macroinvertebrate Community

Approximately 310 days were spent sorting, grouping, and identifying 59,305 organisms. Each organism was handled individually with forceps a minimum of three times during processing and identification. Seasonal organism counts (Appendix E) were highest in spring with a total of 18,388 (31%); summer 17,405 (29%), fall 15,334 (26%), and lastly winter at 8,178 organisms (14%). A total of 5 phyla, 8 classes, 14 orders, and 48 families were identified (Appendix F).

Taxa Richness

Taxa richness was highest during spring (mean 24 taxa) followed by winter (23.6), fall (20.1), and summer (19.3) (Figure 18). During spring the highest number (34) of taxa was observed at R4.3; the minimum value of 18 taxa occurred at R4.1 and R5 (Figure 18). No associations between physical point measurements and taxa richness were found at the habitat scale. At the seasonal scale, an association was found between taxa richness and substrate size (Pearson's $r = -0.664$, Bonferroni's 0.027, $n=40$).

During winter, the site highest in mean taxa richness was R3.1 (mean = 30.6 taxa). No associations were found at the habitat scale with physical point measurements ($n = 5$). At the seasonal scale, no associations between taxa richness and physical point measurements were found ($n = 40$).

During fall, R4.3 was highest in taxa richness (mean = 24.6 taxa). Site R5 had the lowest mean value (14). No associations ($n=5$) were found between taxa richness and point measurements at R5. Site R5.1 was dry during the macroinvertebrate-sampling event. No associations between richness, other community metrics, or point measurements were found at the seasonal level (n=35).

During summer, the highest taxa richness occurred at R4.3 (mean = 26). Site R5.1 was the lowest in taxa richness (mean = 3.8 taxa). At this time, site R5.1 was experiencing channel drying due to drought, and water depths were uniformly low. At the seasonal scale, no associations between taxa richness and point measurements were found (n=40).

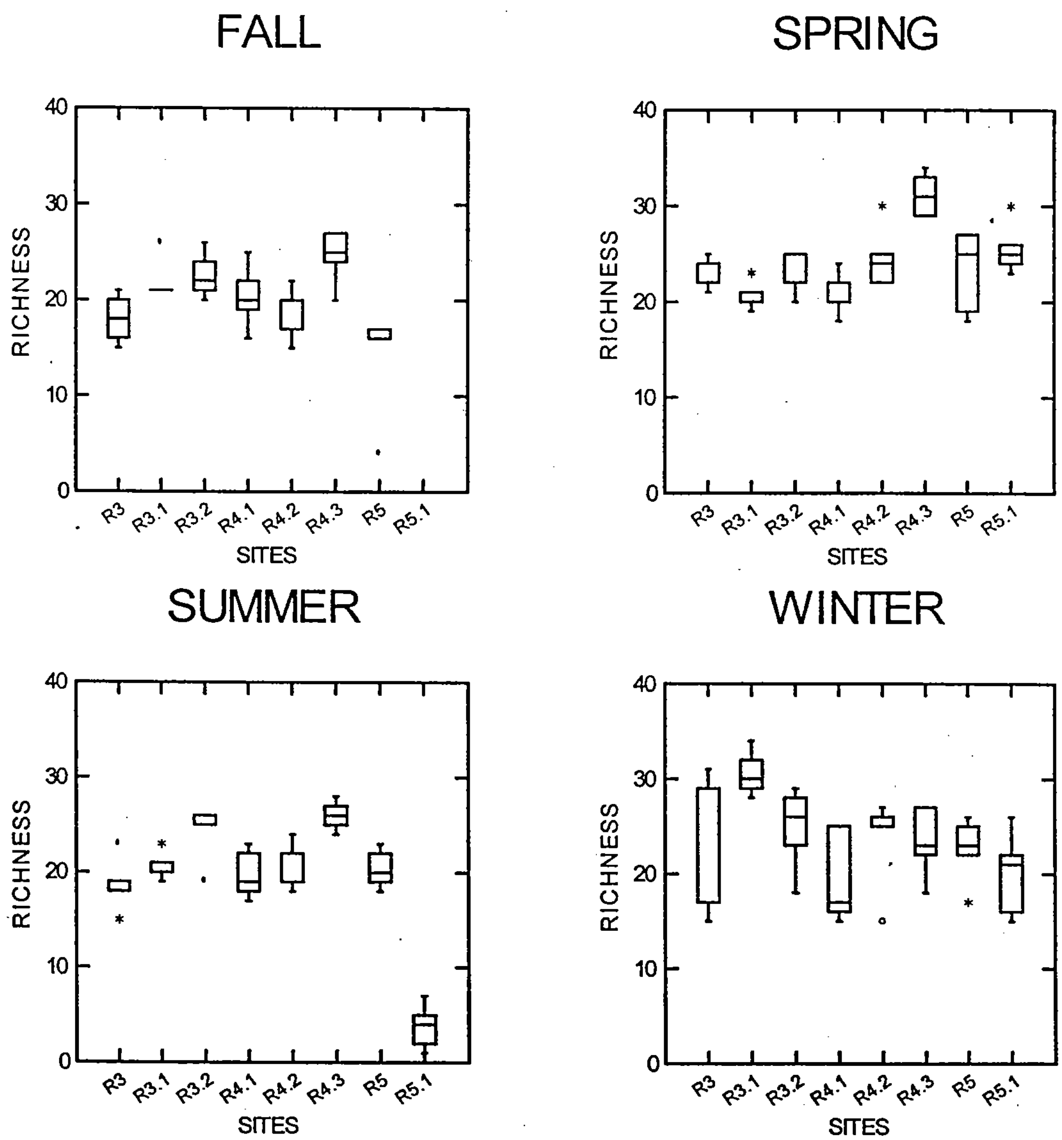

Figure 18. Seasonal macroinvertebrate community characteristics using taxa richness by site with box plot distributions (n = 5 per box).

Shannon's Index of Community Diversity

Winter was highest in diversity among the seasons (mean = 2.424). Site R4.3 had the highest in diversity during winter (2.716) (Figure 19). Lowest in diversity was R5 (1.920). Diversity at R3.2 was found to be related to point depth (Spearman's, $r_s = 0.900$, <0.05, n = 5). No relationships at the season scale were found during winter (n=40).

Spring was second highest in mean diversity (mean = 2.342). Site R4.3 was highest in diversity during spring (2.532), and R.3.2 was the second highest (2.390). R4.2 was the lowest (mean = 2.192). No associations were found at the habitat or seasonal scale for diversity.

Fall was third highest in diversity (mean = 2.182). Site R5 was visually lowest in diversity among the sites. Diversity at R4.1 was found to be associated with bottom velocity (Spearman's, $r_s = 1.000$, <0.05, n = 5), but R4.1 did not appear to be different in diversity from other sites. No other habitat scale associations were found at the other sites for diversity. R5.1 was dry. At the season scale, community diversity and abundance of Corbicula was observed to have a negative relationship (Spearman's $r_s = -0.342$, < 0.05, n=34). Spearman's rank was used at this level because Shannon's index values could not be normalized. This association was made with the abundance of Corbicula removed from the community diversity values.

Summer had the lowest diversity values of all the seasons (mean = 2.160). Site 5.1 was experiencing drought conditions and drying at the time of collection. Site R4.1was highest in average diversity (2.464). Diversity at R4.2 was found to be related to bottom velocity (Spearman's, $r_s = 0.900$, <0.05, n = 5). At the seasonal scale, no relationships were observed.

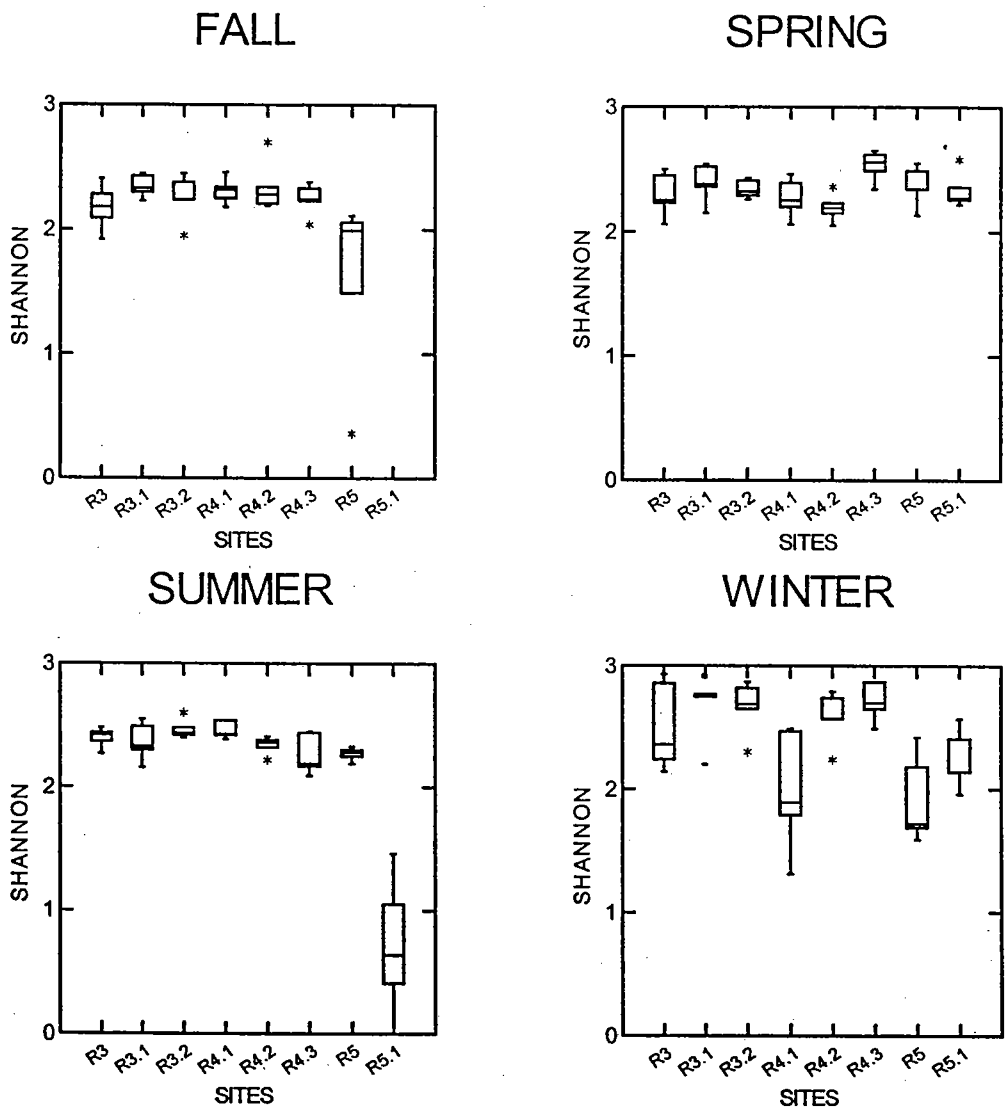

Figure 19. Macroinvertebrate community, seasonal site comparisons as characterized by Shannon's diversity index using box plot distributions (n = 5 per box).

Abundance of Ephemeroptera, Plecoptera, Trichoptera (EPT)

The abundance of EPT was highest during spring (mean = 316.9). Site R5.1 had the highest abundance of EPT (mean = 453.2) (Figure 20). Site R5.1was dry during the fall. The abundance of EPT at two sites, R3.1 and R5, was found to be negatively related to bottom velocity. R3.1 (Spearman's, $r_s = -0.900$, <0.05, n = 5) and R5 (Spearman's, $r_s = -0.900$, <0.05, n = 5) both had positive relationships with increasing velocity.

Fall was the second highest in the abundance of EPT among the seasons (mean = 271.9). Site R4.3 had the highest abundance of EPT among sites (mean = 410.0) and R3.1, below Mill Creek, was lowest (mean = 152.0). Sites R3.2, R4.2, and R5 were also high in the abundance of EPT (means = 409.2, 346.6, and 224.2, respectively). At sites R3, R4.2, and R4.3 positive relationships were noted with the abundance of Corbicula (Spearman's, all $r_s = 0.900$, <0.05, n = 5). At the season level, no relationships were observed with the abundance of EPT and physical habitat or the abundance of Corbicula.

Summer was the third highest among the seasons for EPT (mean = 263.8). Site R3.2 was highest (mean = 525.3), and an association with depth was observed (Spearman's, $r_s = -0.900$, <0.05, n = 5). The next highest site was R5 (mean = 410.4), and R5 was observed to have an association with the abundance of Corbicula (Spearman's, $r_s = 1.000$, <0.05, n = 5). Site R4.3 was the third highest (mean = 369), and at R4.3 abundance of EPT was found to be associated with depth (Spearman's, $r_s = -0.900$, <0.05, n= 5). Site R5.1 was lowest (mean = 1.2) and was experiencing drought conditions.

Winter was lowest in the abundance of EPT among the seasons (mean = 87.0). Site R4.3 was highest for the abundance of EPT (mean = 111.4). The lowest site in EPT was R4.1 (mean = 43.2). Site R3.2 was considered to be low in EPT (mean = 94.4), and an association with the abundance of Corbicula was observed at R3.2 (Spearman's, r_s = 0.900, <0.05, n =5). At the season scale, the abundance of EPT had no associations with physical habitat variables or other community metrics.

The year scale, which was all seasons combined (n = 155), was the largest level tested for associations. At this level, the abundance of EPT (log transformed) was found to be associated with depth (Pearson's, r = -0.450, Bonferroni's 0.000, n =134) and bottom velocity (Pearson's, r = -0.463, Bonferroni's 0.000, n = 134). The decrease in the abundance of EPT was also found, at the year scale, to be negatively associated with elevations in nitrate concentrations collected at the biomonitoring sites (Pearson's, r = -0.839, Bonferroni's 0.000, n = 15). This relationship was not observed at the seasonal level, and the majority of the water-quality collections were taken during spring.

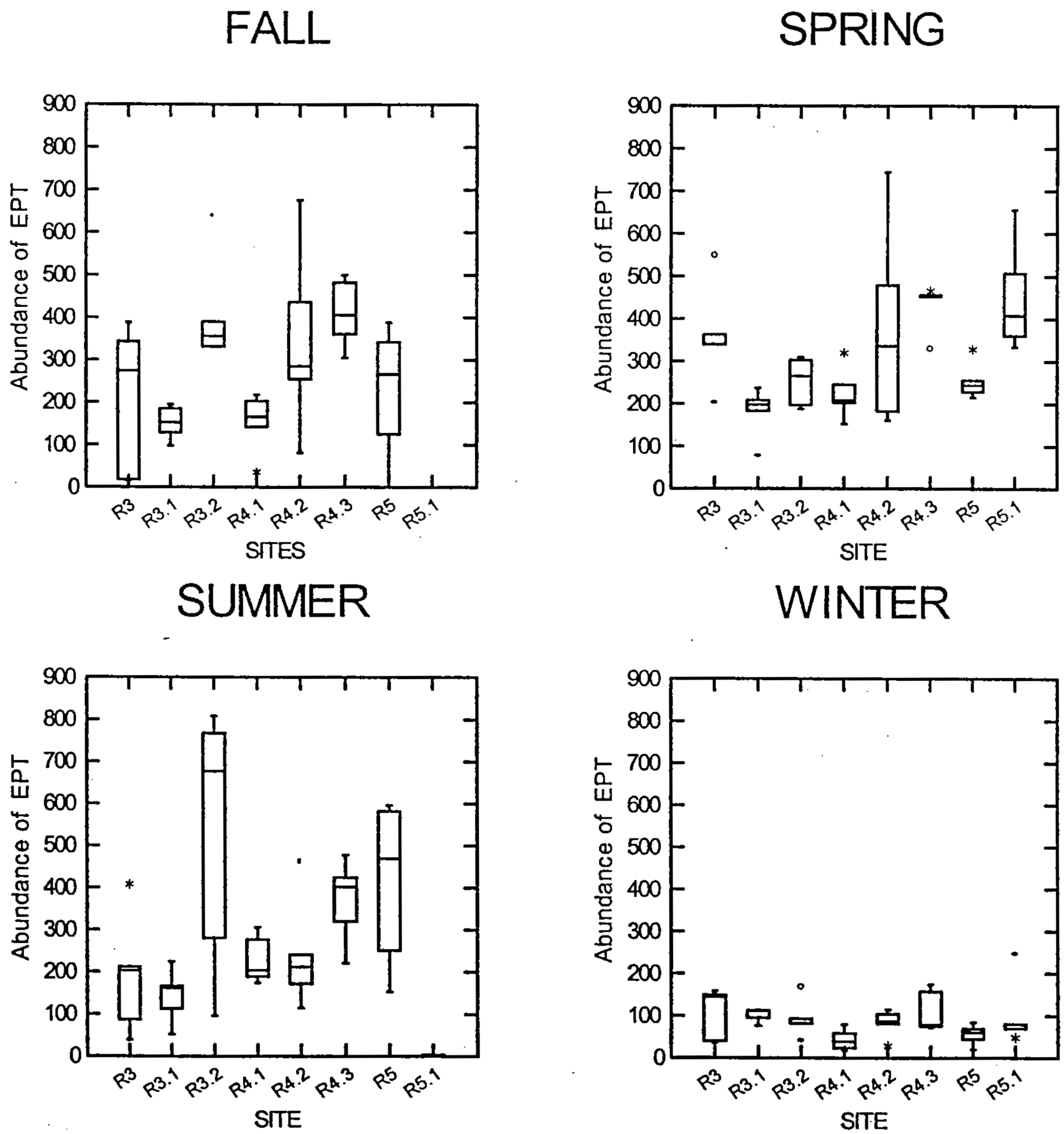

Figure 20. Seasonal macroinvertebrate community comparisons characterized by the abundance of Ephemeroptera-Plecoptera-Trichoptera (EPT) using box plots distributions (n = 5 per site).

Abundance of Diptera

Dipterans are considered to be pollution tolerant and numbers were highest during the spring (mean = 79.2) (Figure 21). Site R4.3 had the highest mean abundance of Diptera (mean = 219.6) among the sites. Next highest was R4.2 (mean = 174.0), and at R4.2, Diptera was found in association with Corbicula (Spearman's, $r_s = -1.000$, <0.05, n = 5). Site R5 was lowest in abundance (mean = 6.8), and the abundance of Diptera was associated with water depth (Spearman's, $r_s = -0.900$, <0.05, n = 5). At the season level, the abundance of Diptera was associated with substrate size (Pearson's, $r_s = -0.521$, Bonferroni's 0.017, n = 36).

Summer was the next highest among the seasons for the abundance of Diptera (mean = 30.2). Site R4.3 had the highest abundance of Diptera among the sites (mean = 90.4). Sites R3.2 and R4.2 were also high in Diptera (means = 55.8 and 54.8, respectively). Site R5.1 was going dry and did not have Dipterans within the samples.

Winter was third highest among the seasons for the abundance of Diptera (mean = 24.7). Site R3.1 had the highest abundance of Diptera (mean = 67.8). The control site, R3, was next highest (mean = 49.4), and abundance of Diptera was found in association with bottom velocity (Spearman's, $r_s = 1.000$, <0.05, n = 5) and Corbicula (Spearman's, $r_s = 1.000$, <0.05, n = 5). Site R4.1 had the lowest abundance of Dipterans (8.2).

Fall was lowest among seasons (mean = 13.7). Site R3.2 had the highest abundance of Diptera (mean = 28.8). Sites R4.3 and R4.2 were the next highest (means = 26.4 and 26, respectively). The abundance of Diptera at R4.3 was observed to be associated with Corbicula (Spearman's, $r_s = -0.900$, <0.05, n =5). Site R5 was lowest in

Diptera (mean = 0.4), and their abundance was found to be associated with substrate size (Spearman's, $r_s = -0.900$, <0.05, $n = 5$).

At the year level, the abundance of Diptera was negatively associated with Corbicula (Pearson's, $r = -0.238$, Bonferroni's 0.017, $n = 134$). Also at this level, the abundance of Diptera and orthophosphate concentration was observed to be associated (Pearson's, $r = 0.427$, Bonferroni's 0.042, $n = 23$).

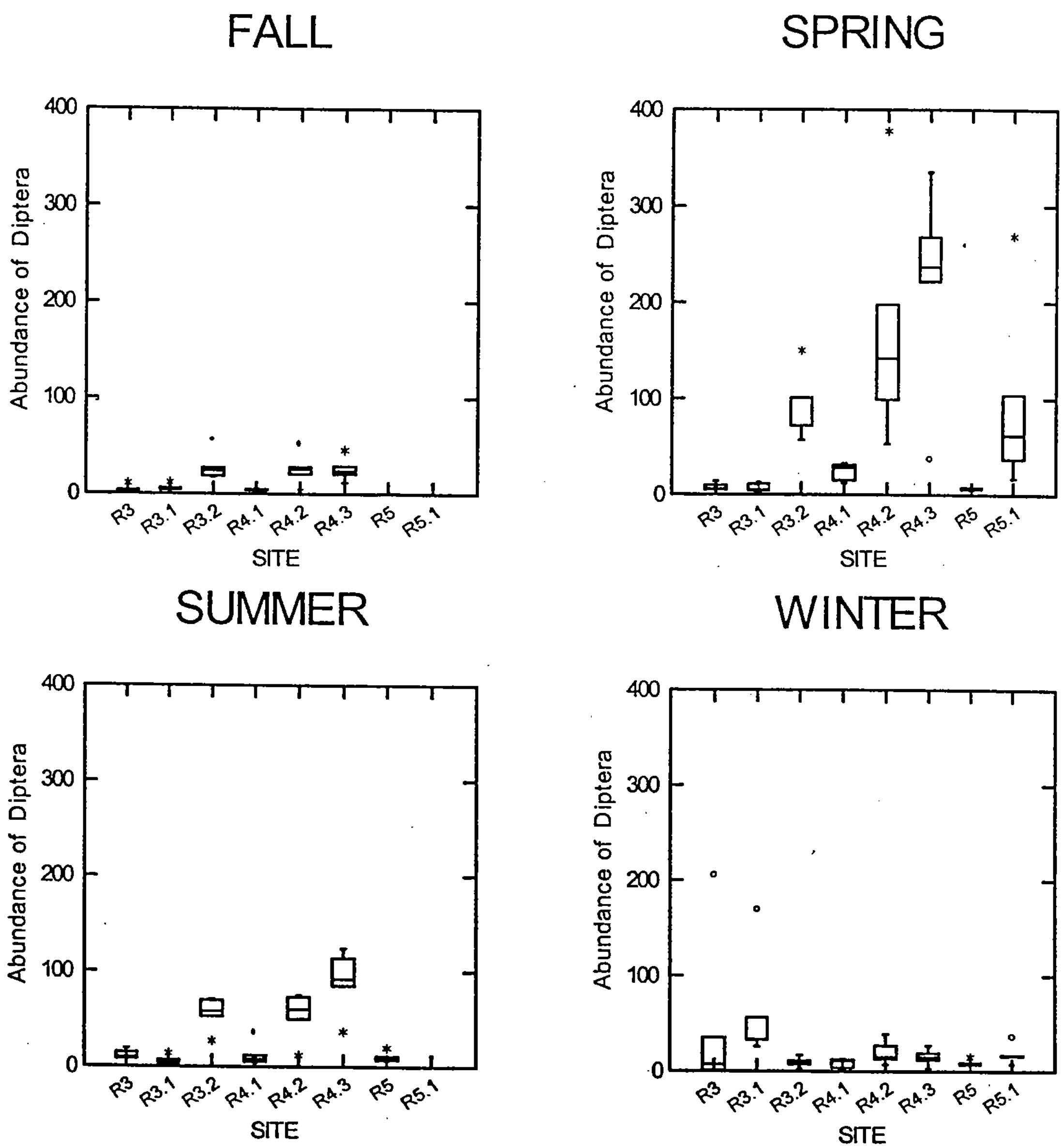

Figure 21. Seasonal macroinvertebrate community comparisons as characterized the abundance of Diptera for each site using box plot distributions of sampling points (n = 5 per box plot).

Abundance of Corbicula

The abundance of Corbicula was highest during summer (mean = 62.0) (Figure 22). Site R5 had the highest abundance of Corbicula (mean = 234.4). The next highest sites were R4.3 and R4.1 (means = 79.8 and 43.0, respectively). Abundance of Corbicula at R4.1 was found to be associated with substrate size (Spearman's, r = -0.921, <0.05, n = 5). Site R5.1 had no Corbicula within the samples. No other associations were found at the riffle or seasonal level with physical habitat variables or other community metrics.

Fall was the next highest season for the abundance of Corbicula (61.4). Among the sites, R5 was again the highest (mean = 240.2), and abundance was observed to be associated with substrate size (Spearman's, r = 1.000, <0.05, n = 5). Site R4.1 was the next highest (mean = 60.6). Site R5.1 was dry. Site R3.2 was the next lowest site (mean = 7.5). At the seasonal level, the surrounding community diversity and the abundance of Corbicula were negatively associated (Spearman's, r = -0.434, <0.05, n = 34). The abundance of Corbicula was removed from the community diversity to avoid creating dependence between the two variables.

Winter was third in abundance of Corbicula among seasons (mean = 37.0). Site R5, similar to summer and fall, had the highest abundance of Corbicula during winter (mean = 150.8). Next highest was R3 (mean = 35.0), which exhibited an association between abundance of Corbicula and bottom velocity (Spearman's, r = 1.000, <0.05, n = 5). Sites R4.1, R4.2, and R4.3 all had positive associations of the abundance of Corbicula with the surrounding community diversity (1.000, 1.000, and 0.900 respectively), and all were significant (<0.05).

Lastly, spring exhibited the lowest abundance of Corbicula (mean = 9.8). All sites were similar with only R3 being slightly higher (mean = 21.4). Associations were observed at R4.2 with the abundance of Corbicula and the surrounding diversity (Spearman's, r = 1.000, <0.05, n = 5). No other associations were found at the riffle level or seasonal level.

Linear multiple regression models were constructed for Corbicula densities for all sites combined by season (Table 5). All regression model strengths were moderate to weak (with R^2 values ranging from 0.592 to 0.294). The fall model resulted in a R^2 of 0.592, the highest amount of variation in the dependent variable explained by the variance in the independent variables. Fall's independent variables ranking from highest to lowest were pH, point depth, bottom velocity, substrate size, and conductivity. Spring had a R^2 value of 0.294, the weakest model. The independent variables in ranking order from highest to lowest were pH, point depth, substrate size, and conductivity. Summer had an R^2 of 0.310 with the following independent variables in order from highest to lowest: point depth, bottom velocity, pH, substrate size, and conductivity. Winter had a R^2 of 0.390 with the following independent variables in order from highest to lowest: point depth, bottom velocity, and substrate size. When all seasonal models were considered, Corbicula's preferred location within riffles can be described as moderately deep with higher bottom velocities and larger substrate size, such as cobble.

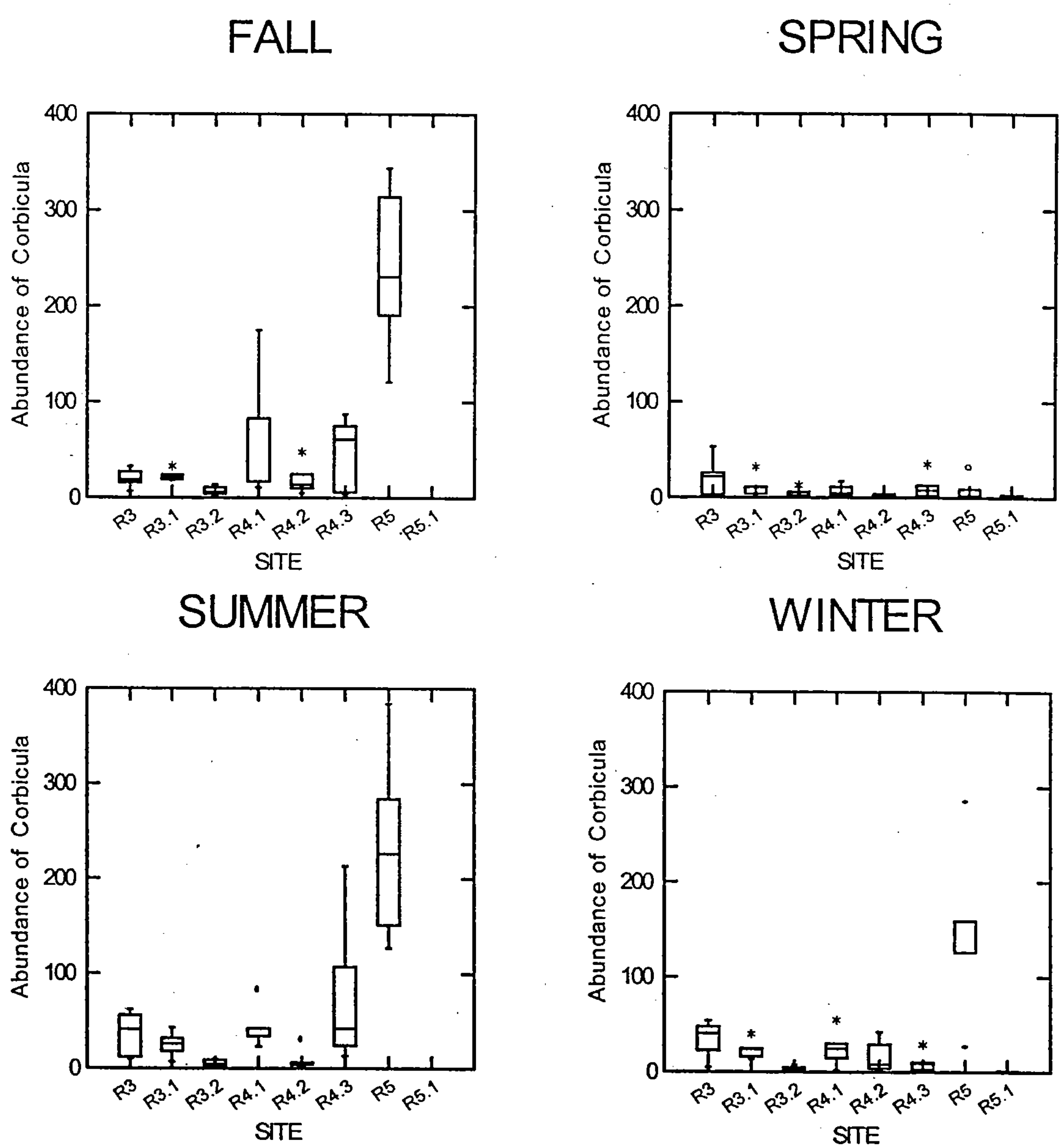

Figure 22. Seasonal macroinvertebrate community comparisons as characterized by % Corbicula for each site using box plot distributions of sampling points (n = 5 per box plot).

Table 5. Multiple regression with Corbicula density as dependent variable and point measurements as independent variables by season.

Season	Dependent	Independent	Coefficient	Tolerance	P-value	R^2	R^2 P-value
Fall	Corbicula Density	Constant	-52.958	0.000	0.000	0.592	0.000
		Point Depth	1.954	0.077	0.617		
		Bottom Velocity	1.007	0.138	0.303		
		Substrate Size	0.029	0.226	0.160		
		Conductivity	-0.005	-0.024	0.852		
		pH	7.035	0.823	0.000		
Spring	Corbicula Density	Constant	-1.632	0.000	0.910	0.294	0.025
		Point Depth	5.742	0.408	0.020		
		Substrate Size	0.013	0.086	0.622		
		Conductivity	-0.013	-0.256	0.160		
		pH	0.590	0.057	0.742		
Summer	Corbicula Density	Constant	0.838	0.000	0.948	0.310	0.046
		Point Depth	3.348	0.137	0.415		
		Bottom Velocity	2.895	0.403	0.015		
		Substrate Size	0.048	0.254	0.137		
		Conductivity	0.019	0.111	0.484		
		pH	-0.752	-0.084	0.589		
Winter	Corbicula Density	Constant	-0.527	0.000	0.764	0.390	0.001
		Point Depth	3.497	0.288	0.076		
		Bottom Velocity	2.119	0.486	0.003		
		Substrate Size	0.011	0.069	0.661		

DISCUSSION

A generalized nutrient cycling model, Nutrient Spiraling, within streams describes the passage of an atomic element from an aquatic phase where it exists as a dissolved available nutrient, through its incorporation into living tissue (biotic phase) and possible passage through several links in the food chain before its eventual release into the water by excretion and/or decomposition (Allan, 1996). In most streams, biological uptake and assimilation of nutrients into organisms is accomplished primarily by autotrophs such as aquatic macrophytes, phytoplankton, and epilithic periphyton. Assimilation rates of dissolved nutrients by periphyton are influenced by the availability of nutrients and physical environmental parameters such as light, space, stream velocity, and water temperature. Aquatic community response to a moderate increase in nutrients typically includes an increase in periphytic density followed by shifts in macroinvertebrate community structure toward herbivory and the grazing functional feeding group (Allan, 1996).

Characterizations of macroinvertebrate assemblages by descriptive community metrics are widely used by stream ecologists (Barbour et al., 1999). Community metrics describe an ecosystem's ability to support niche space, habitat, and food resources that propagate the survival and successful reproduction of existing taxa (Begon et al., 1996). The macroinvertebrate community response to increasing perturbations is expected to result in declines in both taxa richness and diversity (Barbour et al., 1999). Two other diagnostic metrics commonly used by water-quality experts are the abundance of Ephemeroptera-Plecoptera-Trichoptera (EPT) and Diptera taxa groups within a sampled community. Predictions of community response to increasing perturbation, as measured by these two metrics, would be seen as a divergence in the community taxa composition due to differences in tolerances; the abundance of EPT is expected to decline, and Diptera is expected to increase.

Bryant's (1997) investigation of the Buffalo River found declines in macroinvertebrate community taxa richness and diversity that were strongly associated with general increases in nitrate concentrations. He hypothesized that general anthropogenic perturbations existed within the mid-reaches of the Buffalo River and that poorer water-quality played a large component in the degradation of the mid-reach macroinvertebrate community. Based on his findings, this investigation was designed to have an extensive water-quality component, and collection efforts were concentrated in spring, the season that Bryant (1997) observed maximum effect. Although relations between nitrate enrichment and macroinvertebrate community richness and diversity were expected, no such relationships were found during the spring. However, a relationship between decreasing abundance of EPT and increases of nitrate concentrations was observed at the year level of scale, with all the seasons combined. The lack of association between nitrates and community richness and diversity could be due to the differences in water-quality data collection periods. Bryant (1997) used water-quality data from the National Park Service's water-quality monitoring program, and had 12 years of seasonal data from which to derive seasonal concentration means. The current investigation used only data from the biomonitoring stations during the period of study. This study's water-quality samples were collected at both the old and new biomonitoring sites in order to calculate seasonal site concentrations means, thus hydrologic variation over such a limited time period could have introduced confounding error into the correlations.

The tendency of sites with higher nitrates to be lower in abundance of EPT reinforces past observations that eutrophication is likely to be driving macroinvertebrate community composition shifts. Further evidence suggesting nutrient-enrichment effect was provided by the association of increasing abundance of Diptera with increases in orthophosphate concentrations. The divergence of the community between perturbation intolerant and tolerant taxonomic groups indicates that elevated nutrient concentrations are a chronic problem within the mid-reaches and are potentially driving the community species composition change. However, caution should be used when interpreting the possible effect of one disturbance upon a community when more than one disturbance exists and when physical habitat gradients may partially explain differences in abundance levels between sites. For instance, general decreases in the abundance of EPT were observed at locations where increases in nitrate concentrations occurred, a correlation from the year level of scale. At this same level, the abundance of EPT was also potentially effected by gradients within the physical habitat (i.e. increasing depth and bottom velocity). Abundance of Diptera could similarly have been effected by increases in the abundance of <u>Corbicula</u>. Such compounding effects make interpretation of short-term, correlative observations difficult and create a renewed argument for long-term biomonitoring that would help define the natural variation inherent to individual reaches and the river system and possibly make such interpretations easier and more explicit.

The lack of relationships of nutrient concentration with the more conservative community metrics such as richness and diversity suggests that poor water-quality effects upon the macroinvertebrate community might be ephemeral, and a slight improvement in conditions may have occurred. Comparisons of shared water-quality stations (R3, R4, and R5) from the two periods of study (NPS data only) exhibit lower nitrate concentrations at Hasty (R4) and Upper Woolum (R5), indicating that better water-quality conditions were more prevalent during the current period of study. The taxa group chosen by Bryant (1997) to represent perturbation tolerant macroinvertebrates was percent Chironomids, and Bryant's percentages averaged 20 to 50% throughout all seasons. In contrast, the percentages of Diptera, the Order containing Chironomids, during the current study were much lower than the percentages of Chironomidae observed by Bryant (i.e. the percentages of Diptera by season: spring (13.8%), winter (10.7%), summer (6.7), and fall (3.0%)). This indicates that communities had less pollution tolerant taxonomic groups during the current investigation, and that current community conditions are possibly less degraded than previously observed by Bryant.

Comparison of diversity values (Shannon's Index) between shared biomonitoring sites (R3 and R5) exhibited an increase in diversity between the periods of study for one season only (summer). The sample values between the shared biomonitoring sites between the two studies were compared by season using a simple T-test; summer resulted in a significantly higher distribution of diversity values between the two periods of study. Bryant had an average site diversity of 2.15, and the current conditions averaged 2.30 for summer. This indicates, for at least the summer, there was an improvement in the macroinvertebrate community between the two periods of study. The other seasons exhibited no differences in community diversity between sites.

Invasion of non-native species into undisturbed ecosystems is thought to be rare when native species are well adapted (Sax and Brown, 2000). Successful invasions by non-native species typically occur after numerous failed attempts. Highly successful, invasive species are thought to be pre-adapted specifically for human-altered environments (Sax and Brown, 2000). Successful colonization by non-native invaders is enhanced when environments are subject to frequent stochastic disturbances (natural and anthropogenic) to which the native communities are not well adapted and are continuously in various states of recovery. Frequent disturbances disrupt the utilization of resources by native species, thus allowing exotic species to find a niche component that supports a metapopulation of both native and introduced species (Sax and Brown, 2000). Once this metapopulation is established, direct competition for food resources and space between the native and introduced species can occur, which was the case with Corbicula in the Chowan River of North Carolina (Lauritzsen and Samuel, 1983).

The Buffalo River experiences many types of disturbance, both natural and anthropogenic. Seasonal and acute fluctuations in the hydrograph can be extreme (see discussion in the Study Area section). The distributions of Corbicula varied greatly between sites and among the seasons. Summer and fall had the highest abundance of Corbicula, and mean abundance of Corbicula was extremely high at Upper Woolum (234.4 and 240.2, respectively). During fall, the abundance of Corbicula was negatively related to community diversity and the abundance of Diptera, a perturbation tolerant taxonomic group. Negative associations with the surrounding community's diversity and Corbicula could be a result of drought, which is typically at a maximum during this season. Further evidence of this potential drought effect can be seen in the strength of the regression model during fall ($R^2 = 0.592$), when increased depth and bottom velocity were the most critical independent variables for larger densities of Corbicula. This evidence suggests that Corbicula has its greatest effect upon the macroinvertebrate community during periods of low water, and competition for optimal space within the thalwag of the riffle is occurring.

Another factor that may effect densities of Corbicula is hydrologic disturbance. During the period of low flows (summer and fall), the abundance of Corbicula had seasonal means of 62 and 61.4 organisms per sample (705 and 698 clams per m^2). Water levels during this time were not fluctuating and the stage was steadily falling (Figure 7). However, during the period between the fall and winter, one large high-water event occurred, and the mean abundance of Corbicula declined to 37 (420 clams per m^2). Furthermore, in between the winter and spring, four high to medium flood events occurred. After these events, the spring sampling resulted in the lowest mean abundance of Corbicula among the seasons, 9.8 (111 clams per m^2). This suggests that high-water events are a disturbance to populations of Corbicula, and that the cause of the declines in Corbicula is probably substrate mobilization during periods of increased water velocities.

The Upper Woolum Access site (R5) had the highest abundance of Corbicula within the mid-reaches during the investigation by Byrant (1997), who found Corbicula at all river corridor sites with the exception of an extreme headwater site at Dixon Ford. The current investigation found densities of Corbicula at Upper Woolum during fall to range from 343 to 976 clams per m^2. Bryant's (1997) maximum density of Corbicula at Upper Woolum is within the range observed currently (396 clams/ m^2, during fall), but a large increase in mean density was observed between the two studies. Bryant found a mean of 194 clams per m^2 while the current investigation had a mean of 681 clams per m^2 during fall. This 56% increase in mean clam density suggests that conditions at Upper Woolum may be becoming more disturbed. Evidently, Corbicula are dominating the benthic fauna during the low-flow period and are possibly responsible for the general decline in macroinvertebrate community diversity and Diptera levels at riffles with higher densities of Corbicula.

Disturbances occurring at the Upper Woolum Access site are numerous. Mott (1997) found mean nitrate concentrations to be highest at Upper Woolum. The chronic physical habitat disturbances that occur at Upper Woolum include a high-use road crossing, degraded riparian corridors on both banks, free ranging cattle within the river reach, a major recreation access, and a hydrologic anomaly of a losing reach. The losing reach at the Upper Woolum site is immediately upstream from the reach of river that experiences seasonal drying (R5.1), and could play a cryptic role beneficial to Corbicula.

CONCLUSION

The degradation of the mid-reach macroinvertebrate communities of the Buffalo River is a result of a combination of disturbance. Correlative evidence generated during this investigation corroborates the results found by Bryant (1997), and further suggests that declining water-quality and increasing densities of Corbicula are two disturbances that are most likely responsible for shifts in the macroinvertebrate community's species composition within the mid-reaches. Although some evidence suggests that both water-quality and community metric values have slightly improved between the periods of investigation, shared biomonitoring stations had little differences in macroinvertebrate community diversity between the two periods of study, and the densities of Corbicula have dramatically increased, especially at the Upper Woolum river access.

Long-term biomonitoring that examines changes in water-quality, and the abundance of Corbicula, with resulting shifts in macroinvertebrate communities would help better understand the mechanisms that cause macroinvertebrate community change, both natural and anthropogenic. The creation of biomonitoring stations at all NPS-BUFF water-quality sites would document the macroinvertebrate communities within a wide range of nutrient values and physical habitat gradients. Evaluation of the community's condition at sites of varying nutrient levels over several years would establish which sites were chronically low in community diversity and richness.

Long-term monitoring would also provide insight into the resistance and resilience of the macroinvertebrate communities of the tributaries and river. The strengthening of statistical relationships by increasing sample size through long-term observation would better define and separate natural and emerging trends, and would provide Resource Management with empirical data to support more persuasive arguments for improved watershed management and protection. Having macroinvertebrate community data from a wide array of nutrient levels from the tributaries and sites along the main river's corridor would also aid in making predictions in community response and resilience to changes in nutrients concentrations. Sites having chronic higher nutrient values and low community metric values could be used as models to predict the response of macroinvertebrate communities to increasing eutrophication. Using these sites as baseline, prediction of community response within streams that have good water-quality but experiencing increased nutrient loading could then be made with some precision and confidence. Long-term biomonitoring of the macroinvertebrate communities should be considered as a crucial tool in the management of water-quality within the Buffalo National River to assess the degree of community degradation and to predict the loss of the aquatic resources.

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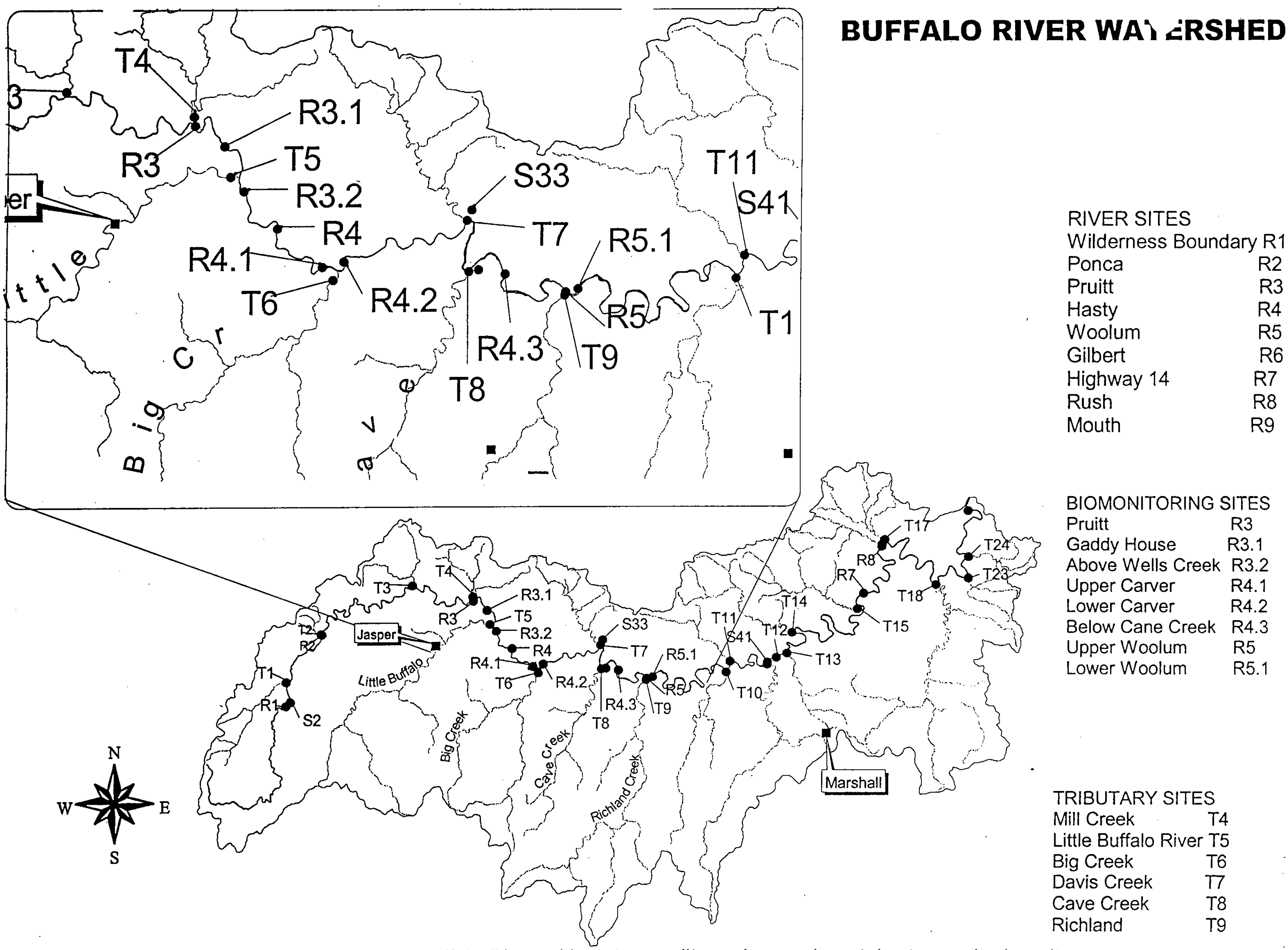

Appendix A. Watershed map of the Buffalo River with water-quality and macroinvertebrate monitoring sites.